

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
20 December 2001 (20.12.2001)

PCT

(10) International Publication Number  
**WO 01/96390 A2**

- (51) International Patent Classification<sup>7</sup>: **C07K 14/47**
- (21) International Application Number: **PCT/US01/18577**
- (22) International Filing Date: **8 June 2001 (08.06.2001)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:  
60/210,821 9 June 2000 (09.06.2000) US  
60/256,571 18 December 2000 (18.12.2000) US  
60/290,240 10 May 2001 (10.05.2001) US
- (71) Applicant (for all designated States except US): **CORIXA CORPORATION** [US/US]; 1124 Columbia Street, Suite 200, Seattle, WA 98104 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **JIANG, Yuqiu** [CN/US]; 5001 S. 232nd Street, Kent, WA 98032 (US). **HEPLER, William, T.** [US/US]; 12034 38th Avenue N.E., Seattle, WA 98125 (US). **CLAPPER, Jonathan, D.** [US/US]; 2149 Dexter Avenue N., #4, Seattle, WA 98109 (US). **WANG, Aijun** [CN/US]; 3106 213th Place S.E., Issaquah, WA 98029 (US). **SECRIST, Heather** [US/US]; 3844 35th Avenue W., Seattle, WA 98199 (US).
- (74) Agents: **POTTER, Jane, E., R.**; Seed Intellectual Property Law Group PLLC, Suite 6300, 701 Fifth Avenue, Seattle, WA 98104-7092 et al. (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:  
— without international search report and to be republished upon receipt of that report.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/96390 A2

(54) Title: COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF COLON CANCER

(57) Abstract: Compositions and methods for the therapy and diagnosis of cancer, such as colon cancer, are disclosed. Compositions may comprise one or more colon tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a colon tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as colon cancer. Diagnostic methods based on detecting a colon tumor protein, or mRNA encoding such a protein, in a sample are also provided.

## COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF COLON CANCER

### TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to therapy and diagnosis of  
5 cancer, such as colon cancer. The invention is more specifically related to polypeptides  
comprising at least a portion of a colon tumor protein, and to polynucleotides encoding  
such polypeptides. Such polypeptides and polynucleotides may be used in vaccines and  
pharmaceutical compositions for prevention and treatment of colon malignancies, and  
for the diagnosis and monitoring of such cancers.

### 10 BACKGROUND OF THE INVENTION

Cancer is a significant health problem throughout the world. Although  
advances have been made in detection and therapy of cancer, no vaccine or other  
universally successful method for prevention or treatment is currently available.  
Current therapies, which are generally based on a combination of chemotherapy or  
15 surgery and radiation, continue to prove inadequate in many patients.

Colon cancer is the second most frequently diagnosed malignancy in the  
United States as well as the second most common cause of cancer death. The five-year  
survival rate for patients with colorectal cancer detected in an early localized stage is  
92%; unfortunately, only 37% of colorectal cancer is diagnosed at this stage. The  
20 survival rate drops to 64% if the cancer is allowed to spread to adjacent organs or  
lymph nodes, and to 7% in patients with distant metastases.

The prognosis of colon cancer is directly related to the degree of  
penetration of the tumor through the bowel wall and the presence or absence of nodal  
involvement, consequently early detection and treatment are especially important.  
25 Currently, diagnosis is aided by the use of screening assays for fecal occult blood,  
sigmoidoscopy, colonoscopy and double contrast barium enemas. Treatment regimens  
are determined by the type and stage of the cancer, and include surgery, radiation  
therapy and/or chemotherapy. Recurrence following surgery (the most common form  
of therapy) is a major problem and is often the ultimate cause of death.

In spite of considerable research into therapies for these and other cancers, colon cancer remains difficult to diagnose and treat effectively. Accordingly, there is a need in the art for improved methods for detecting and treating such cancers. The present invention fulfills these needs and further provides other related advantages.

## 5 SUMMARY OF THE INVENTION

In one aspect, the present invention provides polynucleotide compositions comprising a sequence selected from the group consisting of:

- (a) sequences provided in SEQ ID NOs:1-234, 236, and 244;
- (b) complements of the sequences provided in SEQ ID NOs:1-234,  
10 236, and 244;
- (c) sequences consisting of at least 20, 25, 30, 35, 40, 45, 50, 75 and  
100 contiguous residues of a sequence provided in SEQ ID NOs:1-234, 236, and 244;
- (d) sequences that hybridize to a sequence provided in SEQ ID  
NOs:1-234, 236, and 244, under moderate or highly stringent conditions;
- 15 (e) sequences having at least 75%, 80%, 85%, 90%, 95%, 96%,  
97%, 98% or 99% identity to a sequence of SEQ ID NOs:1-234, 236, and 244;
- (f) degenerate variants of a sequence provided in SEQ ID NOs:1-  
234, 236, and 244.

20 In one preferred embodiment, the polynucleotide compositions of the invention are expressed in at least about 20%, more preferably in at least about 30%, and most preferably in at least about 50% of colon tumor samples tested, at a level that is at least about 2-fold, preferably at least about 5-fold, and most preferably at least about 10-fold higher than that for normal tissues.

25 The present invention, in another aspect, provides polypeptide compositions comprising an amino acid sequence that is encoded by a polynucleotide sequence described above.

The present invention further provides polypeptide compositions comprising an amino acid sequence selected from the group consisting of sequences  
30 recited in SEQ ID NOs:235, 237, and 245.

In certain preferred embodiments, the polypeptides and/or polynucleotides of the present invention are immunogenic, *i.e.*, they are capable of eliciting an immune response, particularly a humoral and/or cellular immune response, as further described herein.

5           The present invention further provides fragments, variants and/or derivatives of the disclosed polypeptide and/or polynucleotide sequences, wherein the fragments, variants and/or derivatives preferably have a level of immunogenic activity of at least about 50%, preferably at least about 70% and more preferably at least about 90% of the level of immunogenic activity of a polypeptide sequence set forth in SEQ  
10 ID NOs:235, 237, and 245 or a polypeptide sequence encoded by a polynucleotide sequence set forth in SEQ ID NOs:1-234, 236, and 244.

The present invention further provides polynucleotides that encode a polypeptide described above, expression vectors comprising such polynucleotides and host cells transformed or transfected with such expression vectors.

15           Within other aspects, the present invention provides pharmaceutical compositions comprising a polypeptide or polynucleotide as described above and a physiologically acceptable carrier.

Within a related aspect of the present invention, the pharmaceutical compositions, *e.g.*, vaccine compositions, are provided for prophylactic or therapeutic  
20 applications. Such compositions generally comprise an immunogenic polypeptide or polynucleotide of the invention and an immunostimulant, such as an adjuvant.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody or antigen-binding fragment thereof that specifically binds to a polypeptide of the present invention, or a fragment thereof; and (b) a physiologically  
25 acceptable carrier.

Within further aspects, the present invention provides pharmaceutical compositions comprising: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) a pharmaceutically acceptable carrier or excipient. Illustrative antigen presenting cells include dendritic cells, macrophages, monocytes, fibroblasts  
30 and B cells.



Within related aspects, pharmaceutical compositions are provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) an immunostimulant.

The present invention further provides, in other aspects, fusion proteins  
5 that comprise at least one polypeptide as described above, as well as polynucleotides encoding such fusion proteins, typically in the form of pharmaceutical compositions, *e.g.*, vaccine compositions, comprising a physiologically acceptable carrier and/or an immunostimulant. The fusions proteins may comprise multiple immunogenic polypeptides or portions/variants thereof, as described herein, and may further comprise  
10 one or more polypeptide segments for facilitating the expression, purification and/or immunogenicity of the polypeptide(s).

Within further aspects, the present invention provides methods for stimulating an immune response in a patient, preferably a T cell response in a human patient, comprising administering a pharmaceutical composition described herein. The  
15 patient may be afflicted with colon cancer, in which case the methods provide treatment for the disease, or patient considered at risk for such a disease may be treated prophylactically.

Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a  
20 patient a pharmaceutical composition as recited above. The patient may be afflicted with colon cancer, in which case the methods provide treatment for the disease, or patient considered at risk for such a disease may be treated prophylactically.

The present invention further provides, within other aspects, methods for removing tumor cells from a biological sample, comprising contacting a biological  
25 sample with T cells that specifically react with a polypeptide of the present invention, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

Within related aspects, methods are provided for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological  
30 sample treated as described above.

Methods are further provided, within other aspects, for stimulating and/or expanding T cells specific for a polypeptide of the present invention, comprising contacting T cells with one or more of: (i) a polypeptide as described above; (ii) a polynucleotide encoding such a polypeptide; and/or (iii) an antigen presenting cell that  
5 expresses such a polypeptide; under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells. Isolated T cell populations comprising T cells prepared as described above are also provided.

Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a  
10 patient an effective amount of a T cell population as described above.

The present invention further provides methods for inhibiting the development of a cancer in a patient, comprising the steps of: (a) incubating CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells isolated from a patient with one or more of: (i) a polypeptide comprising at least an immunogenic portion of polypeptide disclosed herein; (ii) a  
15 polynucleotide encoding such a polypeptide; and (iii) an antigen-presenting cell that expressed such a polypeptide; and (b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient.

20 Within further aspects, the present invention provides methods for determining the presence or absence of a cancer, preferably a colon cancer, in a patient comprising: (a) contacting a biological sample obtained from a patient with a binding agent that binds to a polypeptide as recited above; (b) detecting in the sample an amount of polypeptide that binds to the binding agent; and (c) comparing the amount of  
25 polypeptide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within preferred embodiments, the binding agent is an antibody, more preferably a monoclonal antibody.

The present invention also provides, within other aspects, methods for monitoring the progression of a cancer in a patient. Such methods comprise the steps  
30 of: (a) contacting a biological sample obtained from a patient at a first point in time with a binding agent that binds to a polypeptide as recited above; (b) detecting in the

sample an amount of polypeptide that binds to the binding agent; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polypeptide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the  
5 patient.

The present invention further provides, within other aspects, methods for determining the presence or absence of a cancer in a patient, comprising the steps of: (a) contacting a biological sample, e.g., tumor sample, serum sample, etc., obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a  
10 polypeptide of the present invention; (b) detecting in the sample a level of a polynucleotide, preferably mRNA, that hybridizes to the oligonucleotide; and (c) comparing the level of polynucleotide that hybridizes to the oligonucleotide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within certain embodiments, the amount of mRNA is detected  
15 via polymerase chain reaction using, for example, at least one oligonucleotide primer that hybridizes to a polynucleotide encoding a polypeptide as recited above, or a complement of such a polynucleotide. Within other embodiments, the amount of mRNA is detected using a hybridization technique, employing an oligonucleotide probe that hybridizes to a polynucleotide that encodes a polypeptide as recited above, or a  
20 complement of such a polynucleotide.

In related aspects, methods are provided for monitoring the progression of a cancer in a patient, comprising the steps of: (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a polypeptide of the present invention; (b) detecting in the sample an amount of  
25 a polynucleotide that hybridizes to the oligonucleotide; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polynucleotide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

30 Within further aspects, the present invention provides antibodies, such as monoclonal antibodies, that bind to a polypeptide as described above, as well as

diagnostic kits comprising such antibodies. Diagnostic kits comprising one or more oligonucleotide probes or primers as described above are also provided.

These and other aspects of the present invention will become apparent upon reference to the following detailed description. All references disclosed herein are  
5 hereby incorporated by reference in their entirety as if each was incorporated individually.

#### BRIEF DESCRIPTION OF THE SEQUENCE IDENTIFIERS

SEQ ID NO: 1 is the determined cDNA sequence for 54172.1.

SEQ ID NO: 2 is the determined cDNA sequence for 54104.1 which  
10 shares homology with PAC 75N13 on chromosome Xq21.1.

SEQ ID NO: 3 is the determined cDNA sequence for 53978.1 which shares homology with Glutamine:fructose-6 phosphate amidotransferase.

SEQ ID NO: 4 is the determined cDNA sequence for 54184.1 which shares homology with Colon Kruppel-like factor.

15 SEQ ID NO: 5 is the determined cDNA sequence for 54149.1 which shares homology with cDNA FLJ10461 fis, clone NT2RP1001482.

SEQ ID NO: 6 is the determined cDNA sequence for 54034.1.

SEQ ID NO: 7 is the determined cDNA sequence for 54085.1 which shares homology with Human beta 2 gene.

20 SEQ ID NO: 8 is the determined cDNA sequence for 53948.1 which shares homology with 12p12 BAC RPC111-267J23.

SEQ ID NO: 9 is the determined cDNA sequence for 54026.1 which shares homology with Clone 164F3 on chromosome X2q21.33-23.

25 SEQ ID NO: 10 is the determined cDNA sequence for 53907.1 which shares homology with Lysyl hydroxylase isoform 2.

SEQ ID NO: 11 is the determined cDNA sequence for 54066.1 which shares homology with Mucin 11.

SEQ ID NO: 12 is the determined cDNA sequence for 54017.1 which shares homology with Mucin 11.

SEQ ID NO: 13 is the determined cDNA sequence for 54006.1 which shares homology with Mucin 11.

SEQ ID NO: 14 is the determined cDNA sequence for 53962.1 which shares homology with Epiregulin (EGF family).

5        SEQ ID NO: 15 is the determined cDNA sequence for 54028.1 which shares homology with Mucin 12.

SEQ ID NO: 16 is the determined cDNA sequence for 54166.1 which shares homology with E1A enhancer binding protein.

10       SEQ ID NO: 17 is the determined cDNA sequence for 54174.1 which shares homology with PAC clone RP1-170O19 from 7p15-p21.

SEQ ID NO: 18 is the determined cDNA sequence for 53949.1.

SEQ ID NO: 19 is the determined cDNA sequence for 53898.1.

SEQ ID NO: 20 is the determined cDNA sequence for 54069.1.

15       SEQ ID NO: 21 is the determined cDNA sequence for 54048.1 which shares homology with cDNA FLJ20676 fis, clone KAIA4294.

SEQ ID NO: 22 is the determined cDNA sequence for 54031.1 which shares homology with Chromosome 17, clone hRPC.1171\_1\_10.

SEQ ID NO: 23 is the determined cDNA sequence for 54154.1 which shares homology with Alpha topoisomerase truncated form.

20       SEQ ID NO: 24 is the determined cDNA sequence for 54009.1 which shares homology with Cytokeratin 20.

SEQ ID NO: 25 is the determined cDNA sequence for 54070.1 which shares homology with Erythroblastosis virus oncogene homolog 2.

25       SEQ ID NO: 26 is the determined cDNA sequence for 53998.1 which shares homology with Polyadenylate binding protein II.

SEQ ID NO: 27 is the determined cDNA sequence for 54089.1.

SEQ ID NO: 28 is the determined cDNA sequence for 54182.1 which shares homology with Transforming growth factor-beta induced gene product.

30       SEQ ID NO: 29 is the determined cDNA sequence for 53989.1 which shares homology with GDP-mannose 4,6 dehydratase.

SEQ ID NO: 30 is the determined cDNA sequence for 54181.1.

SEQ ID NO: 31 is the determined cDNA sequence for 54079.1 which shares homology with PAC 75N13 on chromosome Xq21.1.

SEQ ID NO: 32 is the determined cDNA sequence for 54114.1 which shares homology with Mus fork head transcription factor gene.

5 SEQ ID NO: 33 is the determined cDNA sequence for 54160.1 which shares homology with Clone 146H21 on chromosome Xq22.

SEQ ID NO: 34 is the determined cDNA sequence for 54168.1 which shares homology with Glutamine:fructose-6-phosphate amidotransferase.

10 SEQ ID NO: 35 is the determined cDNA sequence for 54078.1 which shares homology with PAC 75N13 on chromosome Xq21.1.

SEQ ID NO: 36 is the determined cDNA sequence for 53900.1 which shares homology with Intestinal peptide-associated transporter HPT-1.

SEQ ID NO: 37 is the determined cDNA sequence for 54147.1.

15 SEQ ID NO: 38 is the determined cDNA sequence for 54033.1 which shares homology with Human proteinase activated receptor-2.

SEQ ID NO: 39 is the determined cDNA sequence for 53908.1 which shares homology with GalNAc-T3 gene.

SEQ ID NO: 40 is the determined cDNA sequence for 54022.1.

20 SEQ ID NO: 41 is the determined cDNA sequence for 54039.1 which shares homology with Constitutive fragile sequence.

SEQ ID NO: 42 is the determined cDNA sequence for 54037.1 which shares homology with CD24 signal transducer gene.

SEQ ID NO: 43 is the determined cDNA sequence for 54129.1 which shares homology with Human c-myb gene.

25 SEQ ID NO: 44 is the determined cDNA sequence for 54054.1 which shares homology with Pyrroline-t-carboxylate synthase long form.

SEQ ID NO: 45 is the determined cDNA sequence for 54055.1 which shares homology with Human zinc finger protein ZNF-139.

30 SEQ ID NO: 46 is the determined cDNA sequence for 54046.1 which shares homology with Gene for membrane cofactor protein.

SEQ ID NO: 47 is the determined cDNA sequence for 54047.1 which shares homology with Colon Kruppel-like factor.

SEQ ID NO: 48 is the determined cDNA sequence for 54040.1 which shares homology with Human capping protein alpha subunit isoform 1.

5 SEQ ID NO: 49 is the determined cDNA sequence for 54035.1 which shares homology with Ig lambda-chain.

SEQ ID NO: 50 is the determined cDNA sequence for 54130.1 which shares homology with Protein tyrosine kinase.

10 SEQ ID NO: 51 is the determined cDNA sequence for 54045.1 which shares homology with cDNA FLJ10610 fis, clone NT2RP2005293.

SEQ ID NO: 52 is the determined cDNA sequence for 54052.1 which shares homology with Human microtubule-associated protein 7.

SEQ ID NO: 53 is the determined cDNA sequence for 54050.1 which shares homology with Human retinoblastoma susceptibility protein.

15 SEQ ID NO: 54 is the determined cDNA sequence for 54051.1 which shares homology with Human reticulocalbin.

SEQ ID NO: 55 is the determined cDNA sequence for 54178.1 which shares homology with Translation initiation factor eIF3 p36 subunit.

20 SEQ ID NO: 56 is the determined cDNA sequence for 54148.1 which shares homology with Human apurinic/aprimidinic-endonuclease.

SEQ ID NO: 57 is the determined cDNA sequence for 54058.1.

SEQ ID NO: 58 is the determined cDNA sequence for 54059.1 which shares homology with Human integral transmembrane protein 1.

25 SEQ ID NO: 59 is the determined cDNA sequence for 54126.1 which shares homology with Human serine kinase.

SEQ ID NO: 60 is the determined cDNA sequence for 54127.1 which shares homology with Human CG1-44 protein.

SEQ ID NO: 61 is the determined cDNA sequence for 54049.1 which shares homology with HADH/NADPH thyroid oxidase p138-tox protein.

30 SEQ ID NO: 62 is the determined cDNA sequence for 54056.1 which shares homology with Human peptide transporter (TAP1) protein.

SEQ ID NO: 63 is the determined cDNA sequence for 54064.1 which shares homology with Clone RP1-39G22 on chromosome 1p32.1-34.3.

SEQ ID NO: 64 is the determined cDNA sequence for 54124.1 which shares homology with Clone Transforming growth factor-beta induced gene product.

5 SEQ ID NO: 65 is the determined cDNA sequence for 54063.1.

SEQ ID NO: 66 is the determined cDNA sequence for 54141.1 which shares homology with Cytokeratin 8.

SEQ ID NO: 67 is the determined cDNA sequence for 54119.1 which shares homology with Human coat protein gamma-cop.

10 SEQ ID NO: 68 is the determined cDNA sequence for 54111.1 which shares homology with Bumetanide-sensitive Na-K-Cl cotransporter.

SEQ ID NO: 69 is the determined cDNA sequence for 54121.1 which shares homology with cDNA FLJ10969 fis, clone PLACE1000909.

15 SEQ ID NO: 70 is the determined cDNA sequence for 54065.1 which shares homology with BAC clone 215O12.

SEQ ID NO: 71 is the determined cDNA sequence for 54060.1 which shares homology with Autoantigen calreticulin.

SEQ ID NO: 72 is the determined cDNA sequence for 54125.1 which shares homology with Human hepatic squalene synthetase.

20 SEQ ID NO: 73 is the determined cDNA sequence for 54143.1 which shares homology with Human RAD21 homolog.

SEQ ID NO: 74 is the determined cDNA sequence for 54139.1 which shares homology with Human MHC class II HLA-DR-alpha.

25 SEQ ID NO: 75 is the determined cDNA sequence for 54137.1 which shares homology with Human Claudin-7.

SEQ ID NO: 76 is the determined cDNA sequence for 54044.1 which shares homology with Ribosome protein S6 kinase 1.

SEQ ID NO: 77 is the determined cDNA sequence for 54042.1 which shares homology with CO-029 tumor associated antigen.

30 SEQ ID NO: 78 is the determined cDNA sequence for 54043.1 which shares homology with KIAA1077 protein.



SEQ ID NO: 79 is the determined cDNA sequence for 54136.1 which shares homology with Human lipocortin II.

SEQ ID NO: 80 is the determined cDNA sequence for 54157.1 which shares homology with PAC 454G6 on chromosome 1q24.

5 SEQ ID NO: 81 is the determined cDNA sequence for 54140.1.

SEQ ID NO: 82 is the determined cDNA sequence for 54120.1.

SEQ ID NO: 83 is the determined cDNA sequence for 54145.1 which shares homology with KIAA0152.

10 SEQ ID NO: 84 is the determined cDNA sequence for 54117.1 which shares homology with Tumor antigen L6.

SEQ ID NO: 85 is the determined cDNA sequence for 54116.1 which shares homology with UDP-N-acetylglucosamine transporter.

SEQ ID NO: 86 is the determined cDNA sequence for 54151.1.

15 SEQ ID NO: 87 is the determined cDNA sequence for 54152.1 which shares homology with Cystine/glutamate transporter.

SEQ ID NO: 88 is the determined cDNA sequence for 54115.1.

SEQ ID NO: 89 is the determined cDNA sequence for 54146.1 which shares homology with GAPDH.

20 SEQ ID NO: 90 is the determined cDNA sequence for 54155.1 which shares homology with cDNA DKFZp586O0118.

SEQ ID NO: 91 is the determined cDNA sequence for 54159.1.

SEQ ID NO: 92 is the determined cDNA sequence for 54020.1 which shares homology with Neutrophil lipocalin.

25 SEQ ID NO: 93 is the determined cDNA sequence for 54169.1 which shares homology with Nuclear matrix protein NRP/B.

SEQ ID NO: 94 is the determined cDNA sequence for 54167.1 which shares homology with CGI-151/KIAA0992 protein.

SEQ ID NO: 95 is the determined cDNA sequence for 54030.1.

SEQ ID NO: 96 is the determined cDNA sequence for 54161.1.

30 SEQ ID NO: 97 is the determined cDNA sequence for 54162.1 which shares homology with Poly A binding protein.

SEQ ID NO: 98 is the determined cDNA sequence for 54163.1 which shares homology with Ribosome protein L13.

SEQ ID NO: 99 is the determined cDNA sequence for 54164.1 which shares homology with Human alpha enolase.

5           SEQ ID NO: 100 is the determined cDNA sequence for 54132.1 which shares homology with Human E-1 enzyme.

SEQ ID NO: 101 is the determined cDNA sequence for 54112.1 which shares homology with cDNA DKFZp58612022.

10           SEQ ID NO: 102 is the determined cDNA sequence for 54133.1 which shares homology with Human ZW10 interactor Zwint.

SEQ ID NO: 103 is the determined cDNA sequence for 54165.1 which shares homology with Bumetanide-sensitive Na-K-Cl cotransporter.

SEQ ID NO: 104 is the determined cDNA sequence for 54158.1 which shares homology with cDNA FLJ10549 fis, clone NT2RP2001976.

15           SEQ ID NO: 105 is the determined cDNA sequence for 54131.1 which shares homology with cDNA DKFZp434C0523.

SEQ ID NO: 106 is the determined cDNA sequence for 54122.1.

SEQ ID NO: 107 is the determined cDNA sequence for 54098.1.

20           SEQ ID NO: 108 is the determined cDNA sequence for 54173.1 which shares homology with NADH-ubiquinone oxidoreductase NDUFS2 subunit.

SEQ ID NO: 109 is the determined cDNA sequence for 54108.1 which shares homology with Phospholipase A2.

SEQ ID NO: 110 is the determined cDNA sequence for 54175.1 which shares homology with cDNA FLJ10610 fis, clone NT2RP2005293.

25           SEQ ID NO: 111 is the determined cDNA sequence for 54179.1 which shares homology with Ig heavy chain variable region.

SEQ ID NO: 112 is the determined cDNA sequence for 54177.1 which shares homology with Protein phosphatase 2C gamma.

30           SEQ ID NO: 113 is the determined cDNA sequence for 54170.1 which shares homology with Cyclin protein.

SEQ ID NO: 114 is the determined cDNA sequence for 54176.1 which shares homology with Transgelin 2 (predicted).

SEQ ID NO: 115 is the determined cDNA sequence for 54180.1 which shares homology with Human GalNAc-T3 gene.

5           SEQ ID NO: 116 is the determined cDNA sequence for 53897.1 which shares homology with cDNA FLJ10884 fis, clone NT2RP4001950.

SEQ ID NO: 117 is the determined cDNA sequence for 54027.1.

SEQ ID NO: 118 is the determined cDNA sequence for 54183.1 which shares homology with Alpha topoisomerase truncated form.

10           SEQ ID NO: 119 is the determined cDNA sequence for 54107.1 which shares homology with KIAA 1289.

SEQ ID NO: 120 is the determined cDNA sequence for 54106.1 which shares homology with AD022 protein.

SEQ ID NO: 121 is the determined cDNA sequence for 53902.1.

15           SEQ ID NO: 122 is the determined cDNA sequence for 53918.1 which shares homology with Chromosome 17, clone hRPK.692\_E\_18.

SEQ ID NO: 123 is the determined cDNA sequence for 53904.1.

SEQ ID NO: 124 is the determined cDNA sequence for 53910.1 which shares homology with cDNA FLJ10823 fis, clone NT2RP4001080.

20           SEQ ID NO: 125 is the determined cDNA sequence for 53903.1 which shares homology with Vector.

SEQ ID NO: 126 is the determined cDNA sequence for 54103.1.

SEQ ID NO: 127 is the determined cDNA sequence for 53917.1 which shares homology with Cytochrome P450 IIIA4.

25           SEQ ID NO: 128 is the determined cDNA sequence for 54004.1 which shares homology with CEA.

SEQ ID NO: 129 is the determined cDNA sequence for 53913.1 which shares homology with Protein phosphatase (KAP1).

SEQ ID NO: 130 is the determined cDNA sequence for 54134.1.

30           SEQ ID NO: 131 is the determined cDNA sequence for 53999.1 which shares homology with Alpha enolase.

SEQ ID NO: 132 is the determined cDNA sequence for 53938.1 which shares homology with Histone deacetylase HD1.

SEQ ID NO: 133 is the determined cDNA sequence for 53939.1 which shares homology with citb\_338\_f\_24, complete sequence.

5       SEQ ID NO: 134 is the determined cDNA sequence for 53928.1 which shares homology with Human squalene epoxidase.

SEQ ID NO: 135 is the determined cDNA sequence for 53914.1 which shares homology with Human aspartyl-tRNA-synthetase alpha-2 subunit.

10       SEQ ID NO: 136 is the determined cDNA sequence for 53915.1 which shares homology with Gamma-actin.

SEQ ID NO: 137 is the determined cDNA sequence for 54101.1 which shares homology with Human AP-mu chain family member mu1B.

SEQ ID NO: 138 is the determined cDNA sequence for 53922.1 which shares homology with Human Cctg mRNA for chaperonin.

15       SEQ ID NO: 139 is the determined cDNA sequence for 54023.1 which shares homology with Chromosome 19.

SEQ ID NO: 140 is the determined cDNA sequence for 53930.1 which shares homology with Human MEGF7.

20       SEQ ID NO: 141 is the determined cDNA sequence for 53921.1 which shares homology with Connexin 26.

SEQ ID NO: 142 is the determined cDNA sequence for 54002.1 which shares homology with Human dipeptidyl peptidase IV.

SEQ ID NO: 143 is the determined cDNA sequence for 54003.1 which shares homology with Chromosome 5 clone CTC-436P18.

25       SEQ ID NO: 144 is the determined cDNA sequence for 54005.1 which shares homology with Human 2-oxoglutarate dehydrogenase.

SEQ ID NO: 145 is the determined cDNA sequence for 53925.1 which shares homology with RHO guanine nucleotide-exchange factor.

30       SEQ ID NO: 146 is the determined cDNA sequence for 53927.1 which shares homology with 12q24 PAC RPC11-261P5.

SEQ ID NO: 147 is the determined cDNA sequence for 54083.1 which shares homology with Human colon mucosa-associated mRNA.

SEQ ID NO: 148 is the determined cDNA sequence for 53937.1.

5      SEQ ID NO: 149 is the determined cDNA sequence for 54074.1 which shares homology with Clone RP4-621F18 on chromosome 1p11.4-21.3.

SEQ ID NO: 150 is the determined cDNA sequence for 54105.1.

SEQ ID NO: 151 is the determined cDNA sequence for 53961.1 which shares homology with Human embryonic lung protein.

SEQ ID NO: 152 is the determined cDNA sequence for 53919.1.

10      SEQ ID NO: 153 is the determined cDNA sequence for 53933.1 which shares homology with Human leukocyte surface protein CD31.

SEQ ID NO: 154 is the determined cDNA sequence for 53972.1 which shares homology with cDNA FLJ10679 fis, clone NT2RP2006565.

SEQ ID NO: 155 is the determined cDNA sequence for 53906.1.

15      SEQ ID NO: 156 is the determined cDNA sequence for 53924.1 which shares homology with Poly A binding protein.

SEQ ID NO: 157 is the determined cDNA sequence for 54144.1.

SEQ ID NO: 158 is the determined cDNA sequence for 54068.1 which shares homology with Cystic fibrosis transmembrane conductance regulator.

20      SEQ ID NO: 159 is the determined cDNA sequence for 53929.1.

SEQ ID NO: 160 is the determined cDNA sequence for 53959.1 which shares homology with KIAA1050.

SEQ ID NO: 161 is the determined cDNA sequence for 53942.1.

25      SEQ ID NO: 162 is the determined cDNA sequence for 53931.1 which shares homology with cDNA FLJ11127 fis, clone PLACE 1006225.

SEQ ID NO: 163 is the determined cDNA sequence for 53935.1 which shares homology with Human set gene.

SEQ ID NO: 164 is the determined cDNA sequence for 54099.1 which shares homology with Human pleckstrin 2.

30      SEQ ID NO: 165 is the determined cDNA sequence for 53943.1 which shares homology with KIAA0965.

SEQ ID NO: 166 is the determined cDNA sequence for 54000.1 which shares homology with Tis 11d gene.

SEQ ID NO: 167 is the determined cDNA sequence for 54100.1 which shares homology with Cyhtokine (GRO-gamma).

5 SEQ ID NO: 168 is the determined cDNA sequence for 53940.1 which shares homology with Human p85Mcm mRNA.

SEQ ID NO: 169 is the determined cDNA sequence for 53941.1 which shares homology with cDNA DKFZp586H0519.

10 SEQ ID NO: 170 is the determined cDNA sequence for 53953.1 which shares homology with SOX9.

SEQ ID NO: 171 is the determined cDNA sequence for 54007.1 which shares homology with VAV-like protein.

SEQ ID NO: 172 is the determined cDNA sequence for 53950.1 which shares homology with NF-E2 related factor 3.

15 SEQ ID NO: 173 is the determined cDNA sequence for 53968.1 which shares homology with cDNA FLJ20127 fis, clone COL06176.

SEQ ID NO: 174 is the determined cDNA sequence for 53945.1.

SEQ ID NO: 175 is the determined cDNA sequence for 54091.1.

20 SEQ ID NO: 176 is the determined cDNA sequence for 54013.1 which shares homology with Human argininosuccinate synthetase.

SEQ ID NO: 177 is the determined cDNA sequence for 54092.1 which shares homology with Human serine kinase.

SEQ ID NO: 178 is the determined cDNA sequence for 54095.1 which shares homology with Clone RP1-155G6 on chromosome 20.

25 SEQ ID NO: 179 is the determined cDNA sequence for 53987.1 which shares homology with Human phospholipase C beta 4.

SEQ ID NO: 180 is the determined cDNA sequence for 53967.1.

SEQ ID NO: 181 is the determined cDNA sequence for 53963.1 which shares homology with VAV-3 protein.

30 SEQ ID NO: 182 is the determined cDNA sequence for 54032.1.

SEQ ID NO: 183 is the determined cDNA sequence for 54067.1 which shares homology with PAC RPCI-1 133G21 map 21q11.1 region D21S190.

SEQ ID NO: 184 is the determined cDNA sequence for 54057.1 which shares homology with Calcium-binding protein S100P.

5           SEQ ID NO: 185 is the determined cDNA sequence for 54135.1 which shares homology with Human leupaxin.

SEQ ID NO: 186 is the determined cDNA sequence for 53969.1 which shares homology with VAV-3 Protein.

SEQ ID NO: 187 is the determined cDNA sequence for 53970.1.

10           SEQ ID NO: 188 is the determined cDNA sequence for 53966.1 which shares homology with hnRNP type A/B protein.

SEQ ID NO: 189 is the determined cDNA sequence for 53995.1 which shares homology with Human cell cycle control gene CDC2.

SEQ ID NO: 190 is the determined cDNA sequence for 54075.1.

15           SEQ ID NO: 191 is the determined cDNA sequence for 54094.1.

SEQ ID NO: 192 is the determined cDNA sequence for 53977.1.

SEQ ID NO: 193 is the determined cDNA sequence for 54123.1 which shares homology with BAC clone RG083M05 from 7q21-7q22.

20           SEQ ID NO: 194 is the determined cDNA sequence for 53960.1 which shares homology with Human STS WI-14644.

SEQ ID NO: 195 is the determined cDNA sequence for 53976.1 which shares homology with Human glutamyl-tRNA synthetase.

SEQ ID NO: 196 is the determined cDNA sequence for 54096.1 which shares homology with Human 26S proteasome-associated pad 1 homolog.

25           SEQ ID NO: 197 is the determined cDNA sequence for 54110.1 which shares homology with Human squalene epoxidase.

SEQ ID NO: 198 is the determined cDNA sequence for 53920.1 which shares homology with Human nuclear chloride ion channel protein.

30           SEQ ID NO: 199 is the determined cDNA sequence for 53979.1 which shares homology with PAC RPCI-1 133G21 map 21q11.1 region D21S190.

SEQ ID NO: 200 is the determined cDNA sequence for 54081.1 which shares homology with PAC clone RP5-1185I7 from 7q11.23-q21.

SEQ ID NO: 201 is the determined cDNA sequence for 54082.1 which shares homology with Human ephrin.

5           SEQ ID NO: 202 is the determined cDNA sequence for 53986.1 which shares homology with cDNA FLJ20673 fis, clone KAIA4464.

SEQ ID NO: 203 is the determined cDNA sequence for 53992.1.

SEQ ID NO: 204 is the determined cDNA sequence for 54016.1.

10           SEQ ID NO: 205 is the determined cDNA sequence for 54018.1 which shares homology with CD9 antigen.

SEQ ID NO: 206 is the determined cDNA sequence for 53985.1 which shares homology with KIAA0715.

SEQ ID NO: 207 is the determined cDNA sequence for 53973.1 which shares homology with Cyclin B.

15           SEQ ID NO: 208 is the determined cDNA sequence for 54012.1 which shares homology with KIAA1225.

SEQ ID NO: 209 is the determined cDNA sequence for 53982.1.

SEQ ID NO: 210 is the determined cDNA sequence for 53988.1 which shares homology with Colon mucosa-associated mRNA.

20           SEQ ID NO: 211 is the determined cDNA sequence for 53990.1 which shares homology with cDNA FLJ20171 fis, clone COL09761.

SEQ ID NO: 212 is the determined cDNA sequence for 53991.1.

SEQ ID NO: 213 is the determined cDNA sequence for 51519.1 which shares homology with CEA.

25           SEQ ID NO: 214 is the determined cDNA sequence for 51507.1 which shares homology with Adenocarcinoma-associated antigen.

SEQ ID NO: 215 is the determined cDNA sequence for 51435.1 which shares homology with Secreted protein XAG.

30           SEQ ID NO: 216 is the determined cDNA sequence for 51425.1 which shares homology with Adenocarcinoma-associated antigen.

SEQ ID NO: 217 is the determined cDNA sequence for 51548.1.



SEQ ID NO: 218 is the determined cDNA sequence for 51430.1 which shares homology with CEA.

SEQ ID NO: 219 is the determined cDNA sequence for 51549.1 which shares homology with CEA.

5           SEQ ID NO: 220 is the determined cDNA sequence for 51439.1 which shares homology with Nonspecific crossreacting antigen.

SEQ ID NO: 221 is the determined cDNA sequence for 51535.1 which shares homology with Neutrophil gelatinase associated lipocalin.

10           SEQ ID NO: 222 is the determined cDNA sequence for 51486.1 which shares homology with Transformation growth factor-beta induced gene product.

SEQ ID NO: 223 is the determined cDNA sequence for 51479.1 which shares homology with Undetermined origin found 5' to NCA mRNA.

SEQ ID NO: 224 is the determined cDNA sequence for 51469.1 which shares homology with Galectin-4.

15           SEQ ID NO: 225 is the determined cDNA sequence for 51470.1 which shares homology with Nonspecific crossreacting antigen.

SEQ ID NO: 226 is the determined cDNA sequence for 51536.1 which shares homology with Secreted protein XAG.

20           SEQ ID NO: 227 is the determined cDNA sequence for 51483.1 which shares homology with Clone 146H21 on chromosome Xq22.

SEQ ID NO: 228 is the determined cDNA sequence for 51522.1 which shares homology with GAPDH.

SEQ ID NO: 229 is the determined cDNA sequence for 51485.1 which shares homology with Mucin 11.

25           SEQ ID NO: 230 is the determined cDNA sequence for 51460.1 which shares homology with Nonspecific crossreacting antigen.

SEQ ID NO: 231 is the determined cDNA sequence for 51458.1 which shares homology with KIAA0517 protein.

30           SEQ ID NO: 232 is the determined cDNA sequence for 51506.1 which shares homology with Surface glycoprotein CD44.

SEQ ID NO: 233 is the determined cDNA sequence for 51440.1 which shares homology with Chromosome 21q22.1, D21S226-AML region.

SEQ ID NO: 234 is the determined cDNA sequence for C907P.

SEQ ID NO: 235 is the amino acid sequence for C907P.

5 SEQ ID NO: 236 is the determine cDNA sequence for Ra12-C915P-f3.

SEQ ID NO: 237 is the amino acid sequence for Ra12-C915P-f3.

SEQ ID NO: 238 is the nucleotide sequence of the AW154 primer.

SEQ ID NO: 239 is the nucleotide sequence of the AW155 primer.

SEQ ID NO: 240 is the nucleotide sequence of the AW156 primer.

10 SEQ ID NO: 241 is the nucleotide sequence of the AW157 primer.

SEQ ID NO: 242 is the nucleotide sequence of the AW158 primer.

SEQ ID NO: 243 is the nucleotide sequence of the AW159 primer.

SEQ ID NO: 244 is the determined full-length cDNA sequence of C915P.

15 SEQ ID NO: 245 is the amino acid sequence encoded by the cDNA sequence set forth in SEQ ID NO:244.

#### DETAILED DESCRIPTION OF THE INVENTION

20 The present invention is directed generally to compositions and their use in the therapy and diagnosis of cancer, particularly colon cancer. As described further below, illustrative compositions of the present invention include, but are not restricted to, polypeptides, particularly immunogenic polypeptides, polynucleotides encoding such polypeptides, antibodies and other binding agents, antigen presenting cells (APCs) and immune system cells (*e.g.*, T cells).

25 The practice of the present invention will employ, unless indicated specifically to the contrary, conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, et al. Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); Maniatis et al. Molecular Cloning:  
30 A Laboratory Manual (1982); DNA Cloning: A Practical Approach, vol. I & II (D.

Glover, ed.); Oligonucleotide Synthesis (N. Gait, ed., 1984); Nucleic Acid Hybridization (B. Hames & S. Higgins, eds., 1985); Transcription and Translation (B. Hames & S. Higgins, eds., 1984); Animal Cell Culture (R. Freshney, ed., 1986); Perbal, A Practical Guide to Molecular Cloning (1984).

5 All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise.

#### 10 POLYPEPTIDE COMPOSITIONS

As used herein, the term "polypeptide" " is used in its conventional meaning, *i.e.*, as a sequence of amino acids. The polypeptides are not limited to a specific length of the product; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide, and such terms may be used interchangeably  
15 herein unless specifically indicated otherwise. This term also does not refer to or exclude post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like, as well as other modifications known in the art, both naturally occurring and non-naturally occurring. A polypeptide may be an entire protein, or a subsequence thereof. Particular polypeptides of interest in the  
20 context of this invention are amino acid subsequences comprising epitopes, *i.e.*, antigenic determinants substantially responsible for the immunogenic properties of a polypeptide and being capable of evoking an immune response.

Particularly illustrative polypeptides of the present invention comprise those encoded by a polynucleotide sequence set forth in any one of SEQ ID NOs:1-234,  
25 236, and 244, or a sequence that hybridizes under moderately stringent conditions, or, alternatively, under highly stringent conditions, to a polynucleotide sequence set forth in any one of SEQ ID NOs:1-234, 236, and 244. Certain other illustrative polypeptides of the invention comprise amino acid sequences as set forth in any one of SEQ ID NOs:235, 237, and 245.

The polypeptides of the present invention are sometimes herein referred to as colon tumor proteins or colon tumor polypeptides, as an indication that their identification has been based at least in part upon their increased levels of expression in colon tumor samples. Thus, a "colon tumor polypeptide" or "colon tumor protein,"  
5 refers generally to a polypeptide sequence of the present invention, or a polynucleotide sequence encoding such a polypeptide, that is expressed in a substantial proportion of colon tumor samples, for example preferably greater than about 20%, more preferably greater than about 30%, and most preferably greater than about 50% or more of colon tumor samples tested, at a level that is at least two fold, and preferably at least five fold,  
10 greater than the level of expression in normal tissues, as determined using a representative assay provided herein. A colon tumor polypeptide sequence of the invention, based upon its increased level of expression in tumor cells, has particular utility both as a diagnostic marker as well as a therapeutic target, as further described below.

15 In certain preferred embodiments, the polypeptides of the invention are immunogenic, *i.e.*, they react detectably within an immunoassay (such as an ELISA or T-cell stimulation assay) with antisera and/or T-cells from a patient with colon cancer. Screening for immunogenic activity can be performed using techniques well known to the skilled artisan. For example, such screens can be performed using methods such as  
20 those described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one illustrative example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, <sup>125</sup>I-labeled Protein A.

25 As would be recognized by the skilled artisan, immunogenic portions of the polypeptides disclosed herein are also encompassed by the present invention. An "immunogenic portion," as used herein, is a fragment of an immunogenic polypeptide of the invention that itself is immunologically reactive (*i.e.*, specifically binds) with the B-cells and/or T-cell surface antigen receptors that recognize the polypeptide.  
30 Immunogenic portions may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press,

1993) and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (*i.e.*, they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well-known techniques.

In one preferred embodiment, an immunogenic portion of a polypeptide of the present invention is a portion that reacts with antisera and/or T-cells at a level that is not substantially less than the reactivity of the full-length polypeptide (*e.g.*, in an ELISA and/or T-cell reactivity assay). Preferably, the level of immunogenic activity of the immunogenic portion is at least about 50%, preferably at least about 70% and most preferably greater than about 90% of the immunogenicity for the full-length polypeptide. In some instances, preferred immunogenic portions will be identified that have a level of immunogenic activity greater than that of the corresponding full-length polypeptide, *e.g.*, having greater than about 100% or 150% or more immunogenic activity.

In certain other embodiments, illustrative immunogenic portions may include peptides in which an N-terminal leader sequence and/or transmembrane domain have been deleted. Other illustrative immunogenic portions will contain a small N- and/or C-terminal deletion (*e.g.*, 1-30 amino acids, preferably 5-15 amino acids), relative to the mature protein.

In another embodiment, a polypeptide composition of the invention may also comprise one or more polypeptides that are immunologically reactive with T cells and/or antibodies generated against a polypeptide of the invention, particularly a polypeptide having an amino acid sequence disclosed herein, or to an immunogenic fragment or variant thereof.

In another embodiment of the invention, polypeptides are provided that comprise one or more polypeptides that are capable of eliciting T cells and/or antibodies that are immunologically reactive with one or more polypeptides described herein, or one or more polypeptides encoded by contiguous nucleic acid sequences contained in the polynucleotide sequences disclosed herein, or immunogenic fragments

or variants thereof, or to one or more nucleic acid sequences which hybridize to one or more of these sequences under conditions of moderate to high stringency.

The present invention, in another aspect, provides polypeptide fragments comprising at least about 5, 10, 15, 20, 25, 50, or 100 contiguous amino acids, or more, including all intermediate lengths, of a polypeptide compositions set forth herein, such as those set forth in SEQ ID NOs:235, 237, and 245, or those encoded by a polynucleotide sequence set forth in a sequence of SEQ ID NOs:1-234, 236, and 244.

In another aspect, the present invention provides variants of the polypeptide compositions described herein. Polypeptide variants generally encompassed by the present invention will typically exhibit at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more identity (determined as described below), along its length, to a polypeptide sequences set forth herein.

In one preferred embodiment, the polypeptide fragments and variants provided by the present invention are immunologically reactive with an antibody and/or T-cell that reacts with a full-length polypeptide specifically set forth herein.

In another preferred embodiment, the polypeptide fragments and variants provided by the present invention exhibit a level of immunogenic activity of at least about 50%, preferably at least about 70%, and most preferably at least about 90% or more of that exhibited by a full-length polypeptide sequence specifically set forth herein.

A polypeptide "variant," as the term is used herein, is a polypeptide that typically differs from a polypeptide specifically disclosed herein in one or more substitutions, deletions, additions and/or insertions. Such variants may be naturally occurring or may be synthetically generated, for example, by modifying one or more of the above polypeptide sequences of the invention and evaluating their immunogenic activity as described herein and/or using any of a number of techniques well known in the art.

For example, certain illustrative variants of the polypeptides of the invention include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other illustrative variants

include variants in which a small portion (*e.g.*, 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein.

In many instances, a variant will contain conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophobic nature of the polypeptide to be substantially unchanged. As described above, modifications may be made in the structure of the polynucleotides and polypeptides of the present invention and still obtain a functional molecule that encodes a variant or derivative polypeptide with desirable characteristics, *e.g.*, with immunogenic characteristics. When it is desired to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, immunogenic variant or portion of a polypeptide of the invention, one skilled in the art will typically change one or more of the codons of the encoding DNA sequence according to Table 1.

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their biological utility or activity.

TABLE 1

Amino Acids			Codons						
Alanine	Ala	A	GCA	GCC	GCG	GCU			
Cysteine	Cys	C	UGC	UGU					
Aspartic acid	Asp	D	GAC	GAU					
Glutamic acid	Glu	E	GAA	GAG					
Phenylalanine	Phe	F	UUC	UUU					
Glycine	Gly	G	GGA	GGC	GGG	GGU			
Histidine	His	H	CAC	CAU					
Isoleucine	Ile	I	AUA	AUC	AUU				
Lysine	Lys	K	AAA	AAG					
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU	
Methionine	Met	M	AUG						
Asparagine	Asn	N	AAC	AAU					
Proline	Pro	P	CCA	CCC	CCG	CCU			
Glutamine	Gln	Q	CAA	CAG					
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU	
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU	
Threonine	Thr	T	ACA	ACC	ACG	ACU			
Valine	Val	V	GUA	GUC	GUG	GUU			
Tryptophan	Trp	W	UGG						
Tyrosine	Tyr	Y	UAC	UAU					

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporated herein by reference). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982). These values are:



isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.* still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within  $\pm 2$  is preferred, those within  $\pm 1$  are particularly preferred, and those within  $\pm 0.5$  are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U. S. Patent 4,554,101 (specifically incorporated herein by reference in its entirety), states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U. S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0  $\pm$  1); glutamate (+3.0  $\pm$  1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5  $\pm$  1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within  $\pm 2$  is preferred, those within  $\pm 1$  are particularly preferred, and those within  $\pm 0.5$  are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those

of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In addition, any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of  
5 flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl-methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

10 Amino acid substitutions may further be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values  
15 include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a  
20 preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydrophobic nature of the polypeptide.

25 As noted above, polypeptides may comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-His), or to enhance binding of the polypeptide to a solid support.  
30 For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

When comparing polypeptide sequences, two sequences are said to be "identical" if the sequence of amino acids in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenies pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) *CABIOS* 5:151-153; Myers, E.W. and Muller W. (1988) *CABIOS* 4:11-17; Robinson, E.D. (1971) *Comb. Theor* 11:105; Saitou, N. Nei, M. (1987) *Mol. Biol. Evol.* 4:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) *Proc. Natl. Acad. Sci. USA* 80:726-730.

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) *Add. APL. Math* 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity methods of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

One preferred example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1977) *Nucl. Acids Res.* 25:3389-3402 and Altschul et al. (1990) *J. Mol. Biol.* 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. For amino acid sequences, a scoring matrix can be used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment.

In one preferred approach, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Within other illustrative embodiments, a polypeptide may be a xenogeneic polypeptide that comprises an polypeptide having substantial sequence identity, as described above, to the human polypeptide (also termed autologous antigen) which served as a reference polypeptide, but which xenogeneic polypeptide is derived from a different, non-human species. One skilled in the art will recognize that "self" antigens are often poor stimulators of CD8+ and CD4+ T-lymphocyte responses,

and therefore efficient immunotherapeutic strategies directed against tumor polypeptides require the development of methods to overcome immune tolerance to particular self tumor polypeptides. For example, humans immunized with prostate protein from a xenogeneic (non human) origin are capable of mounting an immune response against the counterpart human protein, *e.g.* the human prostate tumor protein present on human tumor cells. Accordingly, the present invention provides methods for purifying the xenogeneic form of the tumor proteins set forth herein, such as the polypeptides set forth in SEQ ID NOs:235, 237, and 245, or those encoded by polynucleotide sequences set forth in SEQ ID NOs:1-234, 236, and 244.

Therefore, one aspect of the present invention provides xenogeneic variants of the polypeptide compositions described herein. Such xenogeneic variants generally encompassed by the present invention will typically exhibit at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more identity along their lengths, to a polypeptide sequences set forth herein.

More particularly, the invention is directed to mouse, rat, monkey, porcine and other non-human polypeptides which can be used as xenogeneic forms of human polypeptides set forth herein, to induce immune responses directed against tumor polypeptides of the invention.

Within other illustrative embodiments, a polypeptide may be a fusion polypeptide that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a known tumor protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the polypeptide or to enable the polypeptide to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the polypeptide.

Fusion polypeptides may generally be prepared using standard techniques, including chemical conjugation. Preferably, a fusion polypeptide is

expressed as a recombinant polypeptide, allowing the production of increased levels, relative to a non-fused polypeptide, in an expression system. Briefly, DNA sequences encoding the polypeptide components may be assembled separately, and ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one  
5 polypeptide component is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames of the sequences are in phase. This permits translation into a single fusion polypeptide that retains the biological activity of both component polypeptides.

A peptide linker sequence may be employed to separate the first and  
10 second polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion polypeptide using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a  
15 secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as  
20 linkers include those disclosed in Maratea et al., *Gene* 40:39-46, 1985; Murphy et al., *Proc. Natl. Acad. Sci. USA* 83:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to  
25 separate the functional domains and prevent steric interference.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and  
30 transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

The fusion polypeptide can comprise a polypeptide as described herein together with an unrelated immunogenic protein, such as an immunogenic protein capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (*see, for example, Stoute et al. New Engl. J. Med.*, 5 336:86-91, 1997).

In one preferred embodiment, the immunological fusion partner is derived from a *Mycobacterium* sp., such as a *Mycobacterium tuberculosis*-derived Ra12 fragment. Ra12 compositions and methods for their use in enhancing the expression and/or immunogenicity of heterologous polynucleotide/polypeptide sequences is 10 described in U.S. Patent Application 60/158,585, the disclosure of which is incorporated herein by reference in its entirety. Briefly, Ra12 refers to a polynucleotide region that is a subsequence of a *Mycobacterium tuberculosis* MTB32A nucleic acid. MTB32A is a serine protease of 32 KD molecular weight encoded by a gene in virulent and avirulent strains of *M. tuberculosis*. The nucleotide sequence and amino acid 15 sequence of MTB32A have been described (for example, U.S. Patent Application 60/158,585; *see also, Skeiky et al., Infection and Immun.* (1999) 67:3998-4007, incorporated herein by reference). C-terminal fragments of the MTB32A coding sequence express at high levels and remain as a soluble polypeptides throughout the purification process. Moreover, Ra12 may enhance the immunogenicity of heterologous 20 immunogenic polypeptides with which it is fused. One preferred Ra12 fusion polypeptide comprises a 14 KD C-terminal fragment corresponding to amino acid residues 192 to 323 of MTB32A. Other preferred Ra12 polynucleotides generally comprise at least about 15 consecutive nucleotides, at least about 30 nucleotides, at least about 60 nucleotides, at least about 100 nucleotides, at least about 200 nucleotides, 25 or at least about 300 nucleotides that encode a portion of a Ra12 polypeptide. Ra12 polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence that encodes a Ra12 polypeptide or a portion thereof) or may comprise a variant of such a sequence. Ra12 polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the biological activity of the encoded 30 fusion polypeptide is not substantially diminished, relative to a fusion polypeptide comprising a native Ra12 polypeptide. Variants preferably exhibit at least about 70%

identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native Ra12 polypeptide or a portion thereof.

Within other preferred embodiments, an immunological fusion partner is  
5 derived from protein D, a surface protein of the gram-negative bacterium *Haemophilus influenza B* (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (*e.g.*, the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred  
10 embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in *E. coli* (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemagglutinin). Typically, the N-terminal 81 amino acids are used, although different  
15 fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the *LytA* gene; *Gene* 43:265-292,  
20 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids: useful for expression of fusion proteins. Purification of hybrid proteins  
25 containing the C-LYTA fragment at the amino terminus has been described (*see Biotechnology* 10:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion polypeptide. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

30 Yet another illustrative embodiment involves fusion polypeptides, and the polynucleotides encoding them, wherein the fusion partner comprises a targeting



signal capable of directing a polypeptide to the endosomal/lysosomal compartment, as described in U.S. Patent No. 5,633,234. An immunogenic polypeptide of the invention, when fused with this targeting signal, will associate more efficiently with MHC class II molecules and thereby provide enhanced in vivo stimulation of CD4<sup>+</sup> T-cells specific  
5 for the polypeptide.

Polypeptides of the invention are prepared using any of a variety of well known synthetic and/or recombinant techniques, the latter of which are further described below. Polypeptides, portions and other variants generally less than about 150 amino acids can be generated by synthetic means, using techniques well known to  
10 those of ordinary skill in the art. In one illustrative example, such polypeptides are synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, *J. Am. Chem. Soc.* 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from  
15 suppliers such as Perkin Elmer/Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

In general, polypeptide compositions (including fusion polypeptides) of the invention are isolated. An "isolated" polypeptide is one that is removed from its original environment. For example, a naturally-occurring protein or polypeptide is  
20 isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are also purified, *e.g.*, are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure.

#### POLYNUCLEOTIDE COMPOSITIONS

25 The present invention, in other aspects, provides polynucleotide compositions. The terms "DNA" and "polynucleotide" are used essentially interchangeably herein to refer to a DNA molecule that has been isolated free of total genomic DNA of a particular species. "Isolated," as used herein, means that a polynucleotide is substantially away from other coding sequences, and that the DNA  
30 molecule does not contain large portions of unrelated coding DNA, such as large

chromosomal fragments or other functional genes or polypeptide coding regions. Of course, this refers to the DNA molecule as originally isolated, and does not exclude genes or coding regions later added to the segment by the hand of man.

As will be understood by those skilled in the art, the polynucleotide  
5 compositions of this invention can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the hand of man.

As will be also recognized by the skilled artisan, polynucleotides of the  
10 invention may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules may include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the  
15 present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence that encodes a polypeptide/protein of the invention or a portion thereof) or may comprise a sequence that encodes a variant or derivative, preferably and  
20 immunogenic variant or derivative, of such a sequence.

Therefore, according to another aspect of the present invention, polynucleotide compositions are provided that comprise some or all of a polynucleotide sequence set forth in any one of SEQ ID NOs:1-234, 236, and 244, complements of a polynucleotide sequence set forth in any one of SEQ ID NOs:1-234, 236, and 244, and  
25 degenerate variants of a polynucleotide sequence set forth in any one of SEQ ID NOs:1-234, 236, and 244. In certain preferred embodiments, the polynucleotide sequences set forth herein encode immunogenic polypeptides, as described above.

In other related embodiments, the present invention provides polynucleotide variants having substantial identity to the sequences disclosed herein in  
30 SEQ ID NOs:1-234, 236, and 244, for example those comprising at least 70% sequence identity, preferably at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or

higher, sequence identity compared to a polynucleotide sequence of this invention using the methods described herein, (e.g., BLAST analysis using standard parameters, as described below). One skilled in this art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two  
5 nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

Typically, polynucleotide variants will contain one or more substitutions, additions, deletions and/or insertions, preferably such that the immunogenicity of the polypeptide encoded by the variant polynucleotide is not substantially diminished  
10 relative to a polypeptide encoded by a polynucleotide sequence specifically set forth herein). The term "variants" should also be understood to encompass homologous genes of xenogeneic origin.

In additional embodiments, the present invention provides polynucleotide fragments comprising or consisting of various lengths of contiguous  
15 stretches of sequence identical to or complementary to one or more of the sequences disclosed herein. For example, polynucleotides are provided by this invention that comprise or consist of at least about 10, 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of one or more of the sequences disclosed herein as well as all intermediate lengths there between. It will be readily understood  
20 that "intermediate lengths", in this context, means any length between the quoted values, such as 16, 17, 18, 19, *etc.*; 21, 22, 23, *etc.*; 30, 31, 32, *etc.*; 50, 51, 52, 53, *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers through 200-500; 500-1,000, and the like. A polynucleotide sequence as described here may be extended at one or both ends by additional nucleotides not found in the native sequence.  
25 This additional sequence may consist of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides at either end of the disclosed sequence or at both ends of the disclosed sequence.

In another embodiment of the invention, polynucleotide compositions are provided that are capable of hybridizing under moderate to high stringency  
30 conditions to a polynucleotide sequence provided herein, or a fragment thereof, or a complementary sequence thereof. Hybridization techniques are well known in the art

of molecular biology. For purposes of illustration, suitable moderately stringent conditions for testing the hybridization of a polynucleotide of this invention with other polynucleotides include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-60°C, 5 X SSC, overnight; followed by washing  
5 twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. One skilled in the art will understand that the stringency of hybridization can be readily manipulated, such as by altering the salt content of the hybridization solution and/or the temperature at which the hybridization is performed. For example, in another embodiment, suitable highly stringent hybridization conditions include those  
10 described above, with the exception that the temperature of hybridization is increased, *e.g.*, to 60-65°C or 65-70°C.

In certain preferred embodiments, the polynucleotides described above, *e.g.*, polynucleotide variants, fragments and hybridizing sequences, encode polypeptides that are immunologically cross-reactive with a polypeptide sequence  
15 specifically set forth herein. In other preferred embodiments, such polynucleotides encode polypeptides that have a level of immunogenic activity of at least about 50%, preferably at least about 70%, and more preferably at least about 90% of that for a polypeptide sequence specifically set forth herein.

The polynucleotides of the present invention, or fragments thereof,  
20 regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being  
25 limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative polynucleotide segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful in many implementations of this invention.

30 When comparing polynucleotide sequences, two sequences are said to be "identical" if the sequence of nucleotides in the two sequences is the same when aligned

for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) *Unified Approach to Alignment and Phylogenies* pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) *CABIOS* 5:151-153; Myers, E.W. and Muller W. (1988) *CABIOS* 4:11-17; Robinson, E.D. (1971) *Comb. Theor* 11:105; Santou, N. Nes, M. (1987) *Mol. Biol. Evol.* 4:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) *Proc. Natl. Acad., Sci. USA* 80:726-730.

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) *Add. APL. Math* 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity methods of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

One preferred example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0

algorithms, which are described in Altschul et al. (1977) *Nucl. Acids Res.* 25:3389-3402 and Altschul et al. (1990) *J. Mol. Biol.* 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides of the invention. Software for performing

5 BLAST analyses is publicly available through the National Center for Biotechnology Information. In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). Extension of the word hits in each direction are halted when: the cumulative alignment score falls off

10 by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of

15 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20

20 positions, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical

25 nucleic acid bases occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

It will be appreciated by those of ordinary skill in the art that, as a result

30 of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal

homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous  
5 genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

Therefore, in another embodiment of the invention, a mutagenesis  
10 approach, such as site-specific mutagenesis, is employed for the preparation of immunogenic variants and/or derivatives of the polypeptides described herein. By this approach, specific modifications in a polypeptide sequence can be made through mutagenesis of the underlying polynucleotides that encode them. These techniques provides a straightforward approach to prepare and test sequence variants, for example,  
15 incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the polynucleotide.

Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a  
20 primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Mutations may be employed in a selected polynucleotide sequence to improve, alter, decrease, modify, or otherwise change the properties of the polynucleotide itself, and/or alter the properties, activity, composition, stability, or primary sequence of the encoded polypeptide.

25 In certain embodiments of the present invention, the inventors contemplate the mutagenesis of the disclosed polynucleotide sequences to alter one or more properties of the encoded polypeptide, such as the immunogenicity of a polypeptide vaccine. The techniques of site-specific mutagenesis are well-known in the art, and are widely used to create variants of both polypeptides and polynucleotides.  
30 For example, site-specific mutagenesis is often used to alter a specific portion of a DNA molecule. In such embodiments, a primer comprising typically about 14 to about 25

nucleotides or so in length is employed, with about 5 to about 10 residues on both sides of the junction of the sequence being altered.

As will be appreciated by those of skill in the art, site-specific mutagenesis techniques have often employed a phage vector that exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage are readily commercially-available and their use is generally well-known to those skilled in the art. Double-stranded plasmids are also routinely employed in site directed mutagenesis that eliminates the step of transferring the gene of interest from a plasmid to a phage.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector or melting apart of two strands of a double-stranded vector that includes within its sequence a DNA sequence that encodes the desired peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement.

The preparation of sequence variants of the selected peptide-encoding DNA segments using site-directed mutagenesis provides a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants. Specific details regarding these methods and protocols are found in the teachings of Maloy *et al.*, 1994; Segal, 1976; Prokop and Bajpai, 1991; Kuby, 1994; and Maniatis *et al.*, 1982, each incorporated herein by reference, for that purpose.



As used herein, the term "oligonucleotide directed mutagenesis procedure" refers to template-dependent processes and vector-mediated propagation which result in an increase in the concentration of a specific nucleic acid molecule relative to its initial concentration, or in an increase in the concentration of a detectable  
5 signal, such as amplification. As used herein, the term "oligonucleotide directed mutagenesis procedure" is intended to refer to a process that involves the template-dependent extension of a primer molecule. The term template dependent process refers to nucleic acid synthesis of an RNA or a DNA molecule wherein the sequence of the newly synthesized strand of nucleic acid is dictated by the well-known  
10 rules of complementary base pairing (see, for example, Watson, 1987). Typically, vector mediated methodologies involve the introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of the amplified nucleic acid fragment. Examples of such methodologies are provided by U. S. Patent No. 4,237,224, specifically incorporated herein by reference in its entirety.

15 In another approach for the production of polypeptide variants of the present invention, recursive sequence recombination, as described in U.S. Patent No. 5,837,458, may be employed. In this approach, iterative cycles of recombination and screening or selection are performed to "evolve" individual polynucleotide variants of the invention having, for example, enhanced immunogenic activity.

20 In other embodiments of the present invention, the polynucleotide sequences provided herein can be advantageously used as probes or primers for nucleic acid hybridization. As such, it is contemplated that nucleic acid segments that comprise or consist of a sequence region of at least about a 15 nucleotide long contiguous sequence that has the same sequence as, or is complementary to, a 15 nucleotide long  
25 contiguous sequence disclosed herein will find particular utility. Longer contiguous identical or complementary sequences, e.g., those of about 20, 30, 40, 50, 100, 200, 500, 1000 (including all intermediate lengths) and even up to full length sequences will also be of use in certain embodiments.

The ability of such nucleic acid probes to specifically hybridize to a  
30 sequence of interest will enable them to be of use in detecting the presence of complementary sequences in a given sample. However, other uses are also envisioned,

such as the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

Polynucleotide molecules having sequence regions consisting of contiguous nucleotide stretches of 10-14, 15-20, 30, 50, or even of 100-200 nucleotides  
5 or so (including intermediate lengths as well), identical or complementary to a polynucleotide sequence disclosed herein, are particularly contemplated as hybridization probes for use in, *e.g.*, Southern and Northern blotting. This would allow a gene product, or fragment thereof, to be analyzed, both in diverse cell types and also in various bacterial cells. The total size of fragment, as well as the size of the  
10 complementary stretch(es), will ultimately depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the contiguous complementary region may be varied, such as between about 15 and about 100 nucleotides, but larger contiguous complementarity stretches may be used, according to the length  
15 complementary sequences one wishes to detect.

The use of a hybridization probe of about 15-25 nucleotides in length allows the formation of a duplex molecule that is both stable and selective. Molecules having contiguous complementary sequences over stretches greater than 15 bases in length are generally preferred, though, in order to increase stability and selectivity of  
20 the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 25 contiguous nucleotides, or even longer where desired.

Hybridization probes may be selected from any portion of any of the  
25 sequences disclosed herein. All that is required is to review the sequences set forth herein, or to any continuous portion of the sequences, from about 15-25 nucleotides in length up to and including the full length sequence, that one wishes to utilize as a probe or primer. The choice of probe and primer sequences may be governed by various factors. For example, one may wish to employ primers from towards the termini of the  
30 total sequence.

Small polynucleotide segments or fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer. Also, fragments may be obtained by application of nucleic acid reproduction technology, such as the PCR™  
5 technology of U. S. Patent 4,683,202 (incorporated herein by reference), by introducing selected sequences into recombinant vectors for recombinant production, and by other recombinant DNA techniques generally known to those of skill in the art of molecular biology.

The nucleotide sequences of the invention may be used for their ability  
10 to selectively form duplex molecules with complementary stretches of the entire gene or gene fragments of interest. Depending on the application envisioned, one will typically desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of probe towards target sequence. For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form  
15 the hybrids, *e.g.*, one will select relatively low salt and/or high temperature conditions, such as provided by a salt concentration of from about 0.02 M to about 0.15 M salt at temperatures of from about 50°C to about 70°C. Such selective conditions tolerate little, if any, mismatch between the probe and the template or target strand, and would be particularly suitable for isolating related sequences.

20 Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template, less stringent (reduced stringency) hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ salt conditions such as those of from about 0.15 M to about 0.9 M  
25 salt, at temperatures ranging from about 20°C to about 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus,  
30 hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

According to another embodiment of the present invention, polynucleotide compositions comprising antisense oligonucleotides are provided. Antisense oligonucleotides have been demonstrated to be effective and targeted inhibitors of protein synthesis, and, consequently, provide a therapeutic approach by which a disease can be treated by inhibiting the synthesis of proteins that contribute to the disease. The efficacy of antisense oligonucleotides for inhibiting protein synthesis is well established. For example, the synthesis of polygalacturonase and the muscarine type 2 acetylcholine receptor are inhibited by antisense oligonucleotides directed to their respective mRNA sequences (U. S. Patent 5,739,119 and U. S. Patent 5,759,829). Further, examples of antisense inhibition have been demonstrated with the nuclear protein cyclin, the multiple drug resistance gene (MDG1), ICAM-1, E-selectin, STK-1, striatal GABA<sub>A</sub> receptor and human EGF (Jaskulski *et al.*, Science. 1988 Jun 10;240(4858):1544-6; Vasanthakumar and Ahmed, Cancer Commun. 1989;1(4):225-32; Peris *et al.*, Brain Res Mol Brain Res. 1998 Jun 15;57(2):310-20; U. S. Patent 5,801,154; U.S. Patent 5,789,573; U. S. Patent 5,718,709 and U.S. Patent 5,610,288). Antisense constructs have also been described that inhibit and can be used to treat a variety of abnormal cellular proliferations, e.g. cancer (U. S. Patent 5,747,470; U. S. Patent 5,591,317 and U. S. Patent 5,783,683).

Therefore, in certain embodiments, the present invention provides oligonucleotide sequences that comprise all, or a portion of, any sequence that is capable of specifically binding to polynucleotide sequence described herein, or a complement thereof. In one embodiment, the antisense oligonucleotides comprise DNA or derivatives thereof. In another embodiment, the oligonucleotides comprise RNA or derivatives thereof. In a third embodiment, the oligonucleotides are modified DNAs comprising a phosphorothioated modified backbone. In a fourth embodiment, the oligonucleotide sequences comprise peptide nucleic acids or derivatives thereof. In each case, preferred compositions comprise a sequence region that is complementary, and more preferably substantially-complementary, and even more preferably, completely complementary to one or more portions of polynucleotides disclosed herein. Selection of antisense compositions specific for a given gene sequence is based upon analysis of the chosen target sequence and determination of secondary structure,  $T_m$ ,

binding energy, and relative stability. Antisense compositions may be selected based upon their relative inability to form dimers, hairpins, or other secondary structures that would reduce or prohibit specific binding to the target mRNA in a host cell. Highly preferred target regions of the mRNA, are those which are at or near the AUG  
5 translation initiation codon, and those sequences which are substantially complementary to 5' regions of the mRNA. These secondary structure analyses and target site selection considerations can be performed, for example, using v.4 of the OLIGO primer analysis software and/or the BLASTN 2.0.5 algorithm software (Altschul *et al.*, Nucleic Acids Res. 1997, 25(17):3389-402).

10 The use of an antisense delivery method employing a short peptide vector, termed MPG (27 residues), is also contemplated. The MPG peptide contains a hydrophobic domain derived from the fusion sequence of HIV gp41 and a hydrophilic domain from the nuclear localization sequence of SV40 T-antigen (Morris *et al.*, Nucleic Acids Res. 1997 Jul 15;25(14):2730-6). It has been demonstrated that several  
15 molecules of the MPG peptide coat the antisense oligonucleotides and can be delivered into cultured mammalian cells in less than 1 hour with relatively high efficiency (90%). Further, the interaction with MPG strongly increases both the stability of the oligonucleotide to nuclease and the ability to cross the plasma membrane.

According to another embodiment of the invention, the polynucleotide  
20 compositions described herein are used in the design and preparation of ribozyme molecules for inhibiting expression of the tumor polypeptides and proteins of the present invention in tumor cells. Ribozymes are RNA-protein complexes that cleave nucleic acids in a site-specific fashion. Ribozymes have specific catalytic domains that possess endonuclease activity (Kim and Cech, Proc Natl Acad Sci U S A. 1987  
25 Dec;84(24):8788-92; Forster and Symons, Cell. 1987 Apr 24;49(2):211-20). For example, a large number of ribozymes accelerate phosphoester transfer reactions with a high degree of specificity, often cleaving only one of several phosphoesters in an oligonucleotide substrate (Cech *et al.*, Cell. 1981 Dec;27(3 Pt 2):487-96; Michel and Westhof, J Mol Biol. 1990 Dec 5;216(3):585-610; Reinhold-Hurek and Shub, Nature.  
30 1992 May 14;357(6374):173-6). This specificity has been attributed to the requirement

that the substrate bind via specific base-pairing interactions to the internal guide sequence ("IGS") of the ribozyme prior to chemical reaction.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds *in trans* (and thus can cleave other RNA molecules) under physiological conditions. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over many technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not prevent their action (Woolf *et al.*, Proc Natl Acad Sci U S A. 1992 Aug 15;89(16):7305-9). Thus, the specificity of action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

The enzymatic nucleic acid molecule may be formed in a hammerhead, hairpin, a hepatitis  $\delta$  virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or Neurospora VS RNA motif. Examples of hammerhead motifs are

described by Rossi *et al.* Nucleic Acids Res. 1992 Sep 11;20(17):4559-65. Examples of hairpin motifs are described by Hampel *et al.* (Eur. Pat. Appl. Publ. No. EP 0360257), Hampel and Tritz, Biochemistry 1989 Jun 13;28(12):4929-33; Hampel *et al.*, Nucleic Acids Res. 1990 Jan 25;18(2):299-304 and U. S. Patent 5,631,359. An example of the hepatitis  $\delta$  virus motif is described by Perrotta and Been, Biochemistry. 1992 Dec 1;31(47):11843-52; an example of the RNaseP motif is described by Guerrier-Takada *et al.*, Cell. 1983 Dec;35(3 Pt 2):849-57; Neurospora VS RNA ribozyme motif is described by Collins (Saville and Collins, Cell. 1990 May 18;61(4):685-96; Saville and Collins, Proc Natl Acad Sci U S A. 1991 Oct 1;88(19):8826-30; Collins and Olive, Biochemistry. 1993 Mar 23;32(11):2795-9); and an example of the Group I intron is described in (U. S. Patent 4,987,071). All that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule. Thus the ribozyme constructs need not be limited to specific motifs mentioned herein.

Ribozymes may be designed as described in Int. Pat. Appl. Publ. No. WO 93/23569 and Int. Pat. Appl. Publ. No. WO 94/02595, each specifically incorporated herein by reference) and synthesized to be tested *in vitro* and *in vivo*, as described. Such ribozymes can also be optimized for delivery. While specific examples are provided, those in the art will recognize that equivalent RNA targets in other species can be utilized when necessary.

Ribozyme activity can be optimized by altering the length of the ribozyme binding arms, or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see *e.g.*, Int. Pat. Appl. Publ. No. WO 92/07065; Int. Pat. Appl. Publ. No. WO 93/15187; Int. Pat. Appl. Publ. No. WO 91/03162; Eur. Pat. Appl. Publ. No. 92110298.4; U. S. Patent 5,334,711; and Int. Pat. Appl. Publ. No. WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules), modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan *et al.* (Int. Pat. Appl. Publ. No. WO 94/02595) describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by  
5 incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some indications, ribozymes may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination may be locally delivered by direct inhalation, by direct injection or by use of a catheter, infusion pump or stent.  
10 Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Int. Pat. Appl. Publ. No. WO 94/02595 and Int. Pat. Appl. Publ. No. WO 93/23569, each specifically incorporated  
15 herein by reference.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase  
20 III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, *etc.*) present nearby. Prokaryotic RNA polymerase promoters may also be used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells. Ribozymes  
25 expressed from such promoters have been shown to function in mammalian cells. Such transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated vectors), or viral RNA vectors (such as retroviral, semliki forest virus, sindbis virus vectors).

30 In another embodiment of the invention, peptide nucleic acids (PNAs) compositions are provided. PNA is a DNA mimic in which the nucleobases are



attached to a pseudopeptide backbone (Good and Nielsen, *Antisense Nucleic Acid Drug Dev.* 1997 7(4) 431-37). PNA is able to be utilized in a number methods that traditionally have used RNA or DNA. Often PNA sequences perform better in techniques than the corresponding RNA or DNA sequences and have utilities that are not inherent to RNA or DNA. A review of PNA including methods of making, characteristics of, and methods of using, is provided by Corey (*Trends Biotechnol* 1997 Jun;15(6):224-9). As such, in certain embodiments, one may prepare PNA sequences that are complementary to one or more portions of the ACE mRNA sequence, and such PNA compositions may be used to regulate, alter, decrease, or reduce the translation of ACE-specific mRNA, and thereby alter the level of ACE activity in a host cell to which such PNA compositions have been administered.

PNAs have 2-aminoethyl-glycine linkages replacing the normal phosphodiester backbone of DNA (Nielsen *et al.*, *Science* 1991 Dec 6;254(5037):1497-500; Hanvey *et al.*, *Science*. 1992 Nov 27;258(5087):1481-5; Hyrup and Nielsen, *Bioorg Med Chem.* 1996 Jan;4(1):5-23). This chemistry has three important consequences: firstly, in contrast to DNA or phosphorothioate oligonucleotides, PNAs are neutral molecules; secondly, PNAs are achiral, which avoids the need to develop a stereoselective synthesis; and thirdly, PNA synthesis uses standard Boc or Fmoc protocols for solid-phase peptide synthesis, although other methods, including a modified Merrifield method, have been used.

PNA monomers or ready-made oligomers are commercially available from PerSeptive Biosystems (Framingham, MA). PNA syntheses by either Boc or Fmoc protocols are straightforward using manual or automated protocols (Norton *et al.*, *Bioorg Med Chem.* 1995 Apr;3(4):437-45). The manual protocol lends itself to the production of chemically modified PNAs or the simultaneous synthesis of families of closely related PNAs.

As with peptide synthesis, the success of a particular PNA synthesis will depend on the properties of the chosen sequence. For example, while in theory PNAs can incorporate any combination of nucleotide bases, the presence of adjacent purines can lead to deletions of one or more residues in the product. In expectation of this difficulty, it is suggested that, in producing PNAs with adjacent purines, one should

repeat the coupling of residues likely to be added inefficiently. This should be followed by the purification of PNAs by reverse-phase high-pressure liquid chromatography, providing yields and purity of product similar to those observed during the synthesis of peptides.

- 5                    Modifications of PNAs for a given application may be accomplished by coupling amino acids during solid-phase synthesis or by attaching compounds that contain a carboxylic acid group to the exposed N-terminal amine. Alternatively, PNAs can be modified after synthesis by coupling to an introduced lysine or cysteine. The ease with which PNAs can be modified facilitates optimization for better solubility or
- 10 for specific functional requirements. Once synthesized, the identity of PNAs and their derivatives can be confirmed by mass spectrometry. Several studies have made and utilized modifications of PNAs (for example, Norton *et al.*, *Bioorg Med Chem.* 1995 Apr;3(4):437-45; Petersen *et al.*, *J Pept Sci.* 1995 May-Jun;1(3):175-83; Orum *et al.*, *Biotechniques.* 1995 Sep;19(3):472-80; Footer *et al.*, *Biochemistry.* 1996 Aug
- 15 20;35(33):10673-9; Griffith *et al.*, *Nucleic Acids Res.* 1995 Aug 11;23(15):3003-8; Pardridge *et al.*, *Proc Natl Acad Sci U S A.* 1995 Jun 6;92(12):5592-6; Boffa *et al.*, *Proc Natl Acad Sci U S A.* 1995 Mar 14;92(6):1901-5; Gambacorti-Passerini *et al.*, *Blood.* 1996 Aug 15;88(4):1411-7; Armitage *et al.*, *Proc Natl Acad Sci U S A.* 1997 Nov 11;94(23):12320-5; Seeger *et al.*, *Biotechniques.* 1997 Sep;23(3):512-7). U.S.
- 20 Patent No. 5,700,922 discusses PNA-DNA-PNA chimeric molecules and their uses in diagnostics, modulating protein in organisms, and treatment of conditions susceptible to therapeutics.

- Methods of characterizing the antisense binding properties of PNAs are discussed in Rose (*Anal Chem.* 1993 Dec 15;65(24):3545-9) and Jensen *et al.*
- 25 (Biochemistry. 1997 Apr 22;36(16):5072-7). Rose uses capillary gel electrophoresis to determine binding of PNAs to their complementary oligonucleotide, measuring the relative binding kinetics and stoichiometry. Similar types of measurements were made by Jensen *et al.* using BIAcore™ technology.

- Other applications of PNAs that have been described and will be
- 30 apparent to the skilled artisan include use in DNA strand invasion, antisense inhibition, mutational analysis, enhancers of transcription, nucleic acid purification, isolation of

transcriptionally active genes, blocking of transcription factor binding, genome cleavage, biosensors, *in situ* hybridization, and the like.

#### POLYNUCLEOTIDE IDENTIFICATION, CHARACTERIZATION AND EXPRESSION

Polynucleotides compositions of the present invention may be identified, prepared and/or manipulated using any of a variety of well established techniques (see generally, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989, and other like references). For example, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for tumor-associated expression (*i.e.*, expression that is at least two fold greater in a tumor than in normal tissue, as determined using a representative assay provided herein). Such screens may be performed, for example, using the microarray technology of Affymetrix, Inc. (Santa Clara, CA) according to the manufacturer's instructions (and essentially as described by Schena et al., *Proc. Natl. Acad. Sci. USA* 93:10614-10619, 1996 and Heller et al., *Proc. Natl. Acad. Sci. USA* 94:2150-2155, 1997). Alternatively, polynucleotides may be amplified from cDNA prepared from cells expressing the proteins described herein, such as tumor cells.

Many template dependent processes are available to amplify a target sequences of interest present in a sample. One of the best known amplification methods is the polymerase chain reaction (PCR<sup>TM</sup>) which is described in detail in U.S. Patent Nos. 4,683,195, 4,683,202 and 4,800,159, each of which is incorporated herein by reference in its entirety. Briefly, in PCR<sup>TM</sup>, two primer sequences are prepared which are complementary to regions on opposite complementary strands of the target sequence. An excess of deoxynucleoside triphosphates is added to a reaction mixture along with a DNA polymerase (*e.g.*, *Taq* polymerase). If the target sequence is present in a sample, the primers will bind to the target and the polymerase will cause the primers to be extended along the target sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the target to form reaction products, excess primers will bind to the target and to the reaction product and the process is repeated. Preferably reverse transcription and PCR<sup>TM</sup> amplification procedure may be performed in order to quantify

the amount of mRNA amplified. Polymerase chain reaction methodologies are well known in the art.

Any of a number of other template dependent processes, many of which are variations of the PCR<sup>TM</sup> amplification technique, are readily known and available in the art. Illustratively, some such methods include the ligase chain reaction (referred to as LCR), described, for example, in Eur. Pat. Appl. Publ. No. 320,308 and U.S. Patent No. 4,883,750; Qbeta Replicase, described in PCT Intl. Pat. Appl. Publ. No. PCT/US87/00880; Strand Displacement Amplification (SDA) and Repair Chain Reaction (RCR). Still other amplification methods are described in Great Britain Pat. Appl. No. 2 202 328, and in PCT Intl. Pat. Appl. Publ. No. PCT/US89/01025. Other nucleic acid amplification procedures include transcription-based amplification systems (TAS) (PCT Intl. Pat. Appl. Publ. No. WO 88/10315), including nucleic acid sequence based amplification (NASBA) and 3SR. Eur. Pat. Appl. Publ. No. 329,822 describes a nucleic acid amplification process involving cyclically synthesizing single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA). PCT Intl. Pat. Appl. Publ. No. WO 89/06700 describes a nucleic acid sequence amplification scheme based on the hybridization of a promoter/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. Other amplification methods such as "RACE" (Frohman, 1990), and "one-sided PCR" (Ohara, 1989) are also well-known to those of skill in the art.

An amplified portion of a polynucleotide of the present invention may be used to isolate a full length gene from a suitable library (e.g., a tumor cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (e.g., by nick-translation or end-labeling with <sup>32</sup>P) using well known techniques. A bacterial or bacteriophage library is then generally screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe

(see Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR  
5 using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences can then be assembled into a single contiguous sequence. A full length cDNA  
10 molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, amplification techniques, such as those described above, can be useful for obtaining a full length coding sequence from a partial cDNA sequence. One such amplification technique is inverse PCR (see Triglia et al., *Nucl. Acids Res.* 16:8186, 1988), which uses restriction enzymes to generate a fragment in the  
15 known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of  
20 amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Another such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer,  
25 which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Additional techniques include capture PCR (Lagerstrom et al., *PCR Methods Applic.* 1:111-19, 1991) and walking PCR (Parker et al., *Nucl. Acids. Res.* 19:3055-60, 1991). Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

30 In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as

that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (e.g., NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length DNA sequences may also be obtained by analysis of genomic fragments.

5           In other embodiments of the invention, polynucleotide sequences or fragments thereof which encode polypeptides of the invention, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of a polypeptide in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a  
10 functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express a given polypeptide.

As will be understood by those of skill in the art, it may be advantageous in some instances to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular  
15 prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

Moreover, the polynucleotide sequences of the present invention can be  
20 engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide  
25 sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences may be ligated to a heterologous sequence to  
30 encode a fusion protein. For example, to screen peptide libraries for inhibitors of polypeptide activity, it may be useful to encode a chimeric protein that can be

recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the polypeptide-encoding sequence and the heterologous protein sequence, so that the polypeptide may be cleaved and purified away from the heterologous moiety.

- 5                    Sequences encoding a desired polypeptide may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M. H. et al. (1980) *Nucl. Acids Res. Symp. Ser.* 215-223, Horn, T. et al. (1980) *Nucl. Acids Res. Symp. Ser.* 225-232). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of a polypeptide, or a portion thereof.
- 10    For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J. Y. et al. (1995) *Science* 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer, Palo Alto, CA).

- A newly synthesized peptide may be substantially purified by
- 15    preparative high performance liquid chromatography (e.g., Creighton, T. (1983) *Proteins, Structures and Molecular Principles*, WH Freeman and Co., New York, N.Y.) or other comparable techniques available in the art. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure). Additionally, the amino acid sequence of a polypeptide, or any
- 20    part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

- In order to express a desired polypeptide, the nucleotide sequences encoding the polypeptide, or functional equivalents, may be inserted into appropriate
- 25    expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA
- 30    techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook, J. et al. (1989) *Molecular Cloning*, A

Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y., and Ausubel, F. M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York. N.Y.

A variety of expression vector/host systems may be utilized to contain  
5 and express polynucleotide sequences. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus,  
10 CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems.

The "control elements" or "regulatory sequences" present in an expression vector are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out  
15 transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the pBLUESCRIPT phagemid (Stratagene, La Jolla, Calif.) or  
20 pSPORT1 plasmid (Gibco BRL, Gaithersburg, MD) and the like may be used. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are generally preferred. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding a polypeptide, vectors based on SV40 or EBV may be advantageously used with an appropriate selectable marker.

25 In bacterial systems, any of a number of expression vectors may be selected depending upon the use intended for the expressed polypeptide. For example, when large quantities are needed, for example for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning  
30 and expression vectors such as pBLUESCRIPT (Stratagene), in which the sequence encoding the polypeptide of interest may be ligated into the vector in frame with



sequences for the amino-terminal Met and the subsequent 7 residues of .beta.-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S. M. Schuster (1989) *J. Biol. Chem.* 264:5503-5509); and the like. pGEX Vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion  
5 proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at  
10 will.

In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel et al. (supra) and Grant et al. (1987) *Methods Enzymol.* 153:516-544.

15 In cases where plant expression vectors are used, the expression of sequences encoding polypeptides may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) *EMBO J.* 6:307-311. Alternatively, plant promoters such as the small subunit of  
20 RUBISCO or heat shock promoters may be used (Coruzzi, G. et al. (1984) *EMBO J.* 3:1671-1680; Broglie, R. et al. (1984) *Science* 224:838-843; and Winter, J. et al. (1991) *Results Probl. Cell Differ.* 17:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs, S. or  
25 Murry, L. E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, N.Y.; pp. 191-196).

An insect system may also be used to express a polypeptide of interest. For example, in one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or  
30 in *Trichoplusia* larvae. The sequences encoding the polypeptide may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control

of the polyhedrin promoter. Successful insertion of the polypeptide-encoding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, *S. frugiperda* cells or *Trichoplusia* larvae in which the polypeptide of interest may be expressed  
5 (Engelhard, E. K. et al. (1994) *Proc. Natl. Acad. Sci.* 91 :3224-3227).

In mammalian host cells, a number of viral-based expression systems are generally available. For example, in cases where an adenovirus is used as an expression vector, sequences encoding a polypeptide of interest may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader  
10 sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the polypeptide in infected host cells (Logan, J. and Shenk, T. (1984) *Proc. Natl. Acad. Sci.* 81:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

15 Specific initiation signals may also be used to achieve more efficient translation of sequences encoding a polypeptide of interest. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control  
20 signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic.  
25 The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. et al. (1994) *Results Probl. Cell Differ.* 20:125-162).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the  
30 desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation.

Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, COS, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be  
5 chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is generally preferred. For example, cell lines which stably express a polynucleotide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a  
10 selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed  
15 cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. et al. (1977) *Cell* 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. et al. (1990) *Cell* 22:817-23) genes which can be employed in tk.sup.- or  
20 aprt.sup.- cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. et al. (1980) *Proc. Natl. Acad. Sci.* 77:3567-70); npt, which confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin, F. et al (1981) *J. Mol. Biol.* 150:1-14); and als or pat, which confer resistance to  
25 chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S. C. and R. C. Mulligan (1988) *Proc. Natl. Acad. Sci.* 85:8047-51). The use of visible markers has gained popularity with such markers as  
30 anthocyanins, beta-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the

amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C. A. et al. (1995) *Methods Mol. Biol.* 55:121-131).

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding a polypeptide is inserted within a marker gene sequence, recombinant cells containing sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a polypeptide-encoding sequence under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells that contain and express a desired polynucleotide sequence may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include, for example, membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

A variety of protocols for detecting and measuring the expression of polynucleotide-encoded products, using either polyclonal or monoclonal antibodies specific for the product are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on a given polypeptide may be preferred for some applications, but a competitive binding assay may also be employed. These and other assays are described, among other places, in Hampton, R. et al. (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul. Minn.) and Maddox, D. E. et al. (1983; *J. Exp. Med.* 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences, or any portions

thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of  
5 commercially available kits. Suitable reporter molecules or labels, which may be used include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with a polynucleotide sequence of interest may be cultured under conditions suitable for the expression and recovery of the protein from  
10 cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides of the invention may be designed to contain signal sequences which direct secretion of the encoded polypeptide through a prokaryotic or eukaryotic cell membrane. Other  
15 recombinant constructions may be used to join sequences encoding a polypeptide of interest to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on  
20 immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, Calif.) between the purification domain and the encoded polypeptide may be used to facilitate purification. One such expression vector provides for expression of a fusion  
25 protein containing a polypeptide of interest and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMLAC (immobilized metal ion affinity chromatography) as described in Porath, J. et al. (1992, *Prot. Exp. Purif.* 3:263-281) while the enterokinase cleavage site provides a means for purifying the desired polypeptide from the fusion  
30 protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D. J. et al. (1993; *DNA Cell Biol.* 12:441-453).

In addition to recombinant production methods, polypeptides of the invention, and fragments thereof, may be produced by direct peptide synthesis using solid-phase techniques (Merrifield J. (1963) *J. Am. Chem. Soc.* 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated  
5 synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Alternatively, various fragments may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

#### ANTIBODY COMPOSITIONS, FRAGMENTS THEREOF AND OTHER BINDING AGENTS

10 According to another aspect, the present invention further provides binding agents, such as antibodies and antigen-binding fragments thereof, that exhibit immunological binding to a tumor polypeptide disclosed herein, or to a portion, variant or derivative thereof. An antibody, or antigen-binding fragment thereof, is said to "specifically bind," "immunologically bind," and/or is "immunologically reactive" to a  
15 polypeptide of the invention if it reacts at a detectable level (within, for example, an ELISA assay) with the polypeptide, and does not react detectably with unrelated polypeptides under similar conditions.

Immunological binding, as used in this context, generally refers to the non-covalent interactions of the type which occur between an immunoglobulin  
20 molecule and an antigen for which the immunoglobulin is specific. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant ( $K_d$ ) of the interaction, wherein a smaller  $K_d$  represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of  
25 antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and on geometric parameters that equally influence the rate in both directions. Thus, both the "on rate constant" ( $K_{on}$ ) and the "off rate constant" ( $K_{off}$ ) can be determined by calculation of the concentrations and the actual rates of association and dissociation.  
30 The ratio of  $K_{off}/K_{on}$  enables cancellation of all parameters not related to affinity, and is

thus equal to the dissociation constant  $K_d$ . See, generally, Davies et al. (1990) Annual Rev. Biochem. 59:439-473.

An "antigen-binding site," or "binding portion" of an antibody refers to the part of the immunoglobulin molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy and light chains are referred to as "hypervariable regions" which are interposed between more conserved flanking stretches known as "framework regions," or "FRs". Thus the term "FR" refers to amino acid sequences which are naturally found between and adjacent to hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as "complementarity-determining regions," or "CDRs."

Binding agents may be further capable of differentiating between patients with and without a cancer, such as colon cancer, using the representative assays provided herein. For example, antibodies or other binding agents that bind to a tumor protein will preferably generate a signal indicating the presence of a cancer in at least about 20% of patients with the disease, more preferably at least about 30% of patients. Alternatively, or in addition, the antibody will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without the cancer. To determine whether a binding agent satisfies this requirement, biological samples (e.g., blood, sera, sputum, urine and/or tumor biopsies) from patients with and without a cancer (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. Preferably, a statistically significant number of samples with and without the disease will be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any  
5 of a variety of techniques known to those of ordinary skill in the art. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of  
10 recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (e.g., mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to  
15 a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide  
20 coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the  
25 desired specificity (i.e., reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells  
30 and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid



cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having  
5 high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from  
10 the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

A number of therapeutically useful molecules are known in the art which  
15 comprise antigen-binding sites that are capable of exhibiting immunological binding properties of an antibody molecule. The proteolytic enzyme papain preferentially cleaves IgG molecules to yield several fragments, two of which (the "F(ab)" fragments) each comprise a covalent heterodimer that includes an intact antigen-binding site. The enzyme pepsin is able to cleave IgG molecules to provide several fragments, including  
20 the "F(ab')<sub>2</sub>" fragment which comprises both antigen-binding sites. An "Fv" fragment can be produced by preferential proteolytic cleavage of an IgM, and on rare occasions IgG or IgA immunoglobulin molecule. Fv fragments are, however, more commonly derived using recombinant techniques known in the art. The Fv fragment includes a non-covalent V<sub>H</sub>::V<sub>L</sub> heterodimer including an antigen-binding site which retains much  
25 of the antigen recognition and binding capabilities of the native antibody molecule. Inbar et al. (1972) Proc. Nat. Acad. Sci. USA 69:2659-2662; Hochman et al. (1976) Biochem 15:2706-2710; and Ehrlich et al. (1980) Biochem 19:4091-4096.

A single chain Fv ("sFv") polypeptide is a covalently linked V<sub>H</sub>::V<sub>L</sub> heterodimer which is expressed from a gene fusion including V<sub>H</sub>- and V<sub>L</sub>-encoding  
30 genes linked by a peptide-encoding linker. Huston et al. (1988) Proc. Nat. Acad. Sci. USA 85(16):5879-5883. A number of methods have been described to discern chemical

structures for converting the naturally aggregated--but chemically separated--light and heavy polypeptide chains from an antibody V region into an sFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, *e.g.*, U.S. Pat. Nos. 5,091,513 and 5,132,405, to Huston et al.;  
5 and U.S. Pat. No. 4,946,778, to Ladner et al.

Each of the above-described molecules includes a heavy chain and a light chain CDR set, respectively interposed between a heavy chain and a light chain FR set which provide support to the CDRs and define the spatial relationship of the CDRs relative to each other. As used herein, the term "CDR set" refers to the three  
10 hypervariable regions of a heavy or light chain V region. Proceeding from the N-terminus of a heavy or light chain, these regions are denoted as "CDR1," "CDR2," and "CDR3" respectively. An antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region. A polypeptide comprising a single CDR, (*e.g.*, a CDR1, CDR2 or CDR3) is referred to herein as a  
15 "molecular recognition unit." Crystallographic analysis of a number of antigen-antibody complexes has demonstrated that the amino acid residues of CDRs form extensive contact with bound antigen, wherein the most extensive antigen contact is with the heavy chain CDR3. Thus, the molecular recognition units are primarily responsible for the specificity of an antigen-binding site.

20 As used herein, the term "FR set" refers to the four flanking amino acid sequences which frame the CDRs of a CDR set of a heavy or light chain V region. Some FR residues may contact bound antigen; however, FRs are primarily responsible for folding the V region into the antigen-binding site, particularly the FR residues directly adjacent to the CDRs. Within FRs, certain amino residues and certain structural  
25 features are very highly conserved. In this regard, all V region sequences contain an internal disulfide loop of around 90 amino acid residues. When the V regions fold into a binding-site, the CDRs are displayed as projecting loop motifs which form an antigen-binding surface. It is generally recognized that there are conserved structural regions of FRs which influence the folded shape of the CDR loops into certain "canonical"  
30 structures--regardless of the precise CDR amino acid sequence. Further, certain FR

residues are known to participate in non-covalent interdomain contacts which stabilize the interaction of the antibody heavy and light chains.

A number of "humanized" antibody molecules comprising an antigen-binding site derived from a non-human immunoglobulin have been described, including  
5 chimeric antibodies having rodent V regions and their associated CDRs fused to human constant domains (Winter et al. (1991) *Nature* 349:293-299; Lobuglio et al. (1989) *Proc. Nat. Acad. Sci. USA* 86:4220-4224; Shaw et al. (1987) *J Immunol.* 138:4534-4538; and Brown et al. (1987) *Cancer Res.* 47:3577-3583), rodent CDRs grafted into a human supporting FR prior to fusion with an appropriate human antibody constant  
10 domain (Riechmann et al. (1988) *Nature* 332:323-327; Verhoeyen et al. (1988) *Science* 239:1534-1536; and Jones et al. (1986) *Nature* 321:522-525), and rodent CDRs supported by recombinantly veneered rodent FRs (European Patent Publication No. 519,596, published Dec. 23, 1992). These "humanized" molecules are designed to minimize unwanted immunological response toward rodent antihuman antibody  
15 molecules which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients.

As used herein, the terms "veneered FRs" and "recombinantly veneered FRs" refer to the selective replacement of FR residues from, *e.g.*, a rodent heavy or light chain V region, with human FR residues in order to provide a xenogeneic molecule  
20 comprising an antigen-binding site which retains substantially all of the native FR polypeptide folding structure. Veneering techniques are based on the understanding that the ligand binding characteristics of an antigen-binding site are determined primarily by the structure and relative disposition of the heavy and light chain CDR sets within the antigen-binding surface. Davies et al. (1990) *Ann. Rev. Biochem.* 59:439-473. Thus,  
25 antigen binding specificity can be preserved in a humanized antibody only wherein the CDR structures, their interaction with each other, and their interaction with the rest of the V region domains are carefully maintained. By using veneering techniques, exterior (*e.g.*, solvent-accessible) FR residues which are readily encountered by the immune system are selectively replaced with human residues to provide a hybrid molecule that  
30 comprises either a weakly immunogenic, or substantially non-immunogenic veneered surface.

The process of veneering makes use of the available sequence data for human antibody variable domains compiled by Kabat et al., in Sequences of Proteins of Immunological Interest, 4th ed., (U.S. Dept. of Health and Human Services, U.S. Government Printing Office, 1987), updates to the Kabat database, and other accessible U.S. and foreign databases (both nucleic acid and protein). Solvent accessibilities of V region amino acids can be deduced from the known three-dimensional structure for human and murine antibody fragments. There are two general steps in veneering a murine antigen-binding site. Initially, the FRs of the variable domains of an antibody molecule of interest are compared with corresponding FR sequences of human variable domains obtained from the above-identified sources. The most homologous human V regions are then compared residue by residue to corresponding murine amino acids. The residues in the murine FR which differ from the human counterpart are replaced by the residues present in the human moiety using recombinant techniques well known in the art. Residue switching is only carried out with moieties which are at least partially exposed (solvent accessible), and care is exercised in the replacement of amino acid residues which may have a significant effect on the tertiary structure of V region domains, such as proline, glycine and charged amino acids.

In this manner, the resultant "veneered" murine antigen-binding sites are thus designed to retain the murine CDR residues, the residues substantially adjacent to the CDRs, the residues identified as buried or mostly buried (solvent inaccessible), the residues believed to participate in non-covalent (*e.g.*, electrostatic and hydrophobic) contacts between heavy and light chain domains, and the residues from conserved structural regions of the FRs which are believed to influence the "canonical" tertiary structures of the CDR loops. These design criteria are then used to prepare recombinant nucleotide sequences which combine the CDRs of both the heavy and light chain of a murine antigen-binding site into human-appearing FRs that can be used to transfect mammalian cells for the expression of recombinant human antibodies which exhibit the antigen specificity of the murine antibody molecule.

In another embodiment of the invention, monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives

thereof. Preferred radionuclides include  $^{90}\text{Y}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{211}\text{At}$ , and  $^{212}\text{Bi}$ . Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diphtheria toxin, cholera toxin, gelonin, *Pseudomonas* exotoxin, Shigella toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (*e.g.*, covalently bonded) to a suitable monoclonal antibody either directly or indirectly (*e.g.*, via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (*e.g.*, a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, *e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (*e.g.*, U.S. Patent No. 4,489,710, to Spitler), by irradiation of a

photolabile bond (*e.g.*, U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of derivatized amino acid side chains (*e.g.*, U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (*e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.), and acid-catalyzed hydrolysis (*e.g.*, U.S. Patent No. 4,569,789, to Blattler et al.).

5           It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be  
10 coupled directly to an antibody molecule, or linkers that provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (*e.g.*, U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides  
15 such as aminodextran (*e.g.*, U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (*e.g.*, U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative  
20 radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison et al. discloses representative chelating compounds and their synthesis.

## 25 T CELL COMPOSITIONS

The present invention, in another aspect, provides T cells specific for a tumor polypeptide disclosed herein, or for a variant or derivative thereof. Such cells may generally be prepared *in vitro* or *ex vivo*, using standard procedures. For example, T cells may be isolated from bone marrow, peripheral blood, or a fraction of bone  
30 marrow or peripheral blood of a patient, using a commercially available cell separation

system, such as the Isolex™ System, available from Nexell Therapeutics, Inc. (Irvine, CA; see also U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243). Alternatively, T cells may be derived from related or unrelated humans, non-human mammals, cell lines or cultures.

5 T cells may be stimulated with a polypeptide, polynucleotide encoding a polypeptide and/or an antigen presenting cell (APC) that expresses such a polypeptide. Such stimulation is performed under conditions and for a time sufficient to permit the generation of T cells that are specific for the polypeptide of interest. Preferably, a tumor polypeptide or polynucleotide of the invention is present within a delivery  
10 vehicle, such as a microsphere, to facilitate the generation of specific T cells.

T cells are considered to be specific for a polypeptide of the present invention if the T cells specifically proliferate, secrete cytokines or kill target cells coated with the polypeptide or expressing a gene encoding the polypeptide. T cell specificity may be evaluated using any of a variety of standard techniques. For  
15 example, within a chromium release assay or proliferation assay, a stimulation index of more than two fold increase in lysis and/or proliferation, compared to negative controls, indicates T cell specificity. Such assays may be performed, for example, as described in Chen et al., *Cancer Res.* 54:1065-1070, 1994. Alternatively, detection of the proliferation of T cells may be accomplished by a variety of known techniques. For  
20 example, T cell proliferation can be detected by measuring an increased rate of DNA synthesis (e.g., by pulse-labeling cultures of T cells with tritiated thymidine and measuring the amount of tritiated thymidine incorporated into DNA). Contact with a tumor polypeptide (100 ng/ml - 100 µg/ml, preferably 200 ng/ml - 25 µg/ml) for 3 - 7 days will typically result in at least a two fold increase in proliferation of the T cells.  
25 Contact as described above for 2-3 hours should result in activation of the T cells, as measured using standard cytokine assays in which a two fold increase in the level of cytokine release (e.g., TNF or IFN-γ) is indicative of T cell activation (see Coligan et al., *Current Protocols in Immunology*, vol. 1, Wiley Interscience (Greene 1998)). T cells that have been activated in response to a tumor polypeptide, polynucleotide or  
30 polypeptide-expressing APC may be CD4<sup>+</sup> and/or CD8<sup>+</sup>. Tumor polypeptide-specific T cells may be expanded using standard techniques. Within preferred embodiments, the T

cells are derived from a patient, a related donor or an unrelated donor, and are administered to the patient following stimulation and expansion.

For therapeutic purposes, CD4<sup>+</sup> or CD8<sup>+</sup> T cells that proliferate in response to a tumor polypeptide, polynucleotide or APC can be expanded in number either *in vitro* or *in vivo*. Proliferation of such T cells *in vitro* may be accomplished in a variety of ways. For example, the T cells can be re-exposed to a tumor polypeptide, or a short peptide corresponding to an immunogenic portion of such a polypeptide, with or without the addition of T cell growth factors, such as interleukin-2, and/or stimulator cells that synthesize a tumor polypeptide. Alternatively, one or more T cells that proliferate in the presence of the tumor polypeptide can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution.

#### T CELL RECEPTOR COMPOSITIONS

The T cell receptor (TCR) consists of 2 different, highly variable polypeptide chains, termed the T-cell receptor  $\alpha$  and  $\beta$  chains, that are linked by a disulfide bond (Janeway, Travers, Walport. *Immunobiology*. Fourth Ed., 148-159. Elsevier Science Ltd/Garland Publishing. 1999). The  $\alpha/\beta$  heterodimer complexes with the invariant CD3 chains at the cell membrane. This complex recognizes specific antigenic peptides bound to MHC molecules. The enormous diversity of TCR specificities is generated much like immunoglobulin diversity, through somatic gene rearrangement. The  $\beta$  chain genes contain over 50 variable (V), 2 diversity (D), over 10 joining (J) segments, and 2 constant region segments (C). The  $\alpha$  chain genes contain over 70 V segments, and over 60 J segments but no D segments, as well as one C segment. During T cell development in the thymus, the D to J gene rearrangement of the  $\beta$  chain occurs, followed by the V gene segment rearrangement to the DJ. This functional VDJ $\beta$  exon is transcribed and spliced to join to a C $\beta$ . For the  $\alpha$  chain, a V $\alpha$  gene segment rearranges to a J $\alpha$  gene segment to create the functional exon that is then transcribed and spliced to the C $\alpha$ . Diversity is further increased during the recombination process by the random addition of P and N-nucleotides between the V, D, and J segments of the  $\beta$  chain and between the V and J segments in the  $\alpha$  chain



(Janeway, Travers, Walport. *Immunobiology*. Fourth Ed., 98 and 150. Elsevier Science Ltd/Garland Publishing. 1999).

The present invention, in another aspect, provides TCRs specific for a colon tumor polypeptide disclosed herein, or for a variant or derivative thereof. In accordance with the present invention, polynucleotide and amino acid sequences are provided for the V-J or V-D-J junctional regions or parts thereof for the alpha and beta chains of the T-cell receptor which recognize tumor polypeptides described herein. In general, this aspect of the invention relates to T-cell receptors which recognize or bind tumor polypeptides presented in the context of MHC. In a preferred embodiment the tumor antigens recognized by the T-cell receptors comprise a polypeptide of the present invention. For example, cDNA encoding a TCR specific for a colon tumor peptide can be isolated from T cells specific for a tumor polypeptide using standard molecular biological and recombinant DNA techniques.

This invention further includes the T-cell receptors or analogs thereof having substantially the same function or activity as the T-cell receptors of this invention which recognize or bind tumor polypeptides. Such receptors include, but are not limited to, a fragment of the receptor, or a substitution, addition or deletion mutant of a T-cell receptor provided herein. This invention also encompasses polypeptides or peptides that are substantially homologous to the T-cell receptors provided herein or that retain substantially the same activity. The term "analog" includes any protein or polypeptide having an amino acid residue sequence substantially identical to the T-cell receptors provided herein in which one or more residues, preferably no more than 5 residues, more preferably no more than 25 residues have been conservatively substituted with a functionally similar residue and which displays the functional aspects of the T-cell receptor as described herein.

The present invention further provides for suitable mammalian host cells, for example, non-specific T cells, that are transfected with a polynucleotide encoding TCRs specific for a polypeptide described herein, thereby rendering the host cell specific for the polypeptide. The  $\alpha$  and  $\beta$  chains of the TCR may be contained on separate expression vectors or alternatively, on a single expression vector that also contains an internal ribosome entry site (IRES) for cap-independent translation of the gene downstream of the IRES. Said host cells expressing TCRs specific for the

polypeptide may be used, for example, for adoptive immunotherapy of colon cancer as discussed further below.

In further aspects of the present invention, cloned TCRs specific for a polypeptide recited herein may be used in a kit for the diagnosis of colon cancer. For example, the nucleic acid sequence or portions thereof, of colon tumor-specific TCRs can be used as probes or primers for the detection of expression of the rearranged genes encoding the specific TCR in a biological sample. Therefore, the present invention further provides for an assay for detecting messenger RNA or DNA encoding the TCR specific for a polypeptide.

## 10 PHARMACEUTICAL COMPOSITIONS

In additional embodiments, the present invention concerns formulation of one or more of the polynucleotide, polypeptide, T-cell, TCR, and/or antibody compositions disclosed herein in pharmaceutically-acceptable carriers for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy.

It will be understood that, if desired, a composition as disclosed herein may be administered in combination with other agents as well, such as, *e.g.*, other proteins or polypeptides or various pharmaceutically-active agents. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The compositions may thus be delivered along with various other agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized as described herein. Likewise, such compositions may further comprise substituted or derivatized RNA or DNA compositions.

Therefore, in another aspect of the present invention, pharmaceutical compositions are provided comprising one or more of the polynucleotide, polypeptide, antibody, TCR, and/or T-cell compositions described herein in combination with a physiologically acceptable carrier. In certain preferred embodiments, the pharmaceutical compositions of the invention comprise immunogenic polynucleotide

and/or polypeptide compositions of the invention for use in prophylactic and therapeutic vaccine applications. Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Generally, such compositions will comprise one or more  
5 polynucleotide and/or polypeptide compositions of the present invention in combination with one or more immunostimulants.

It will be apparent that any of the pharmaceutical compositions described herein can contain pharmaceutically acceptable salts of the polynucleotides and polypeptides of the invention. Such salts can be prepared, for example, from  
10 pharmaceutically acceptable non-toxic bases, including organic bases (e.g., salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (e.g., sodium, potassium, lithium, ammonium, calcium and magnesium salts).

In another embodiment, illustrative immunogenic compositions, e.g., vaccine compositions, of the present invention comprise DNA encoding one or more of  
15 the polypeptides as described above, such that the polypeptide is generated *in situ*. As noted above, the polynucleotide may be administered within any of a variety of delivery systems known to those of ordinary skill in the art. Indeed, numerous gene delivery techniques are well known in the art, such as those described by Rolland, *Crit. Rev. Therap. Drug Carrier Systems* 15:143-198, 1998, and references cited therein.  
20 Appropriate polynucleotide expression systems will, of course, contain the necessary regulatory DNA regulatory sequences for expression in a patient (such as a suitable promoter and terminating signal). Alternatively, bacterial delivery systems may involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope.

25 Therefore, in certain embodiments, polynucleotides encoding immunogenic polypeptides described herein are introduced into suitable mammalian host cells for expression using any of a number of known viral-based systems. In one illustrative embodiment, retroviruses provide a convenient and effective platform for gene delivery systems. A selected nucleotide sequence encoding a polypeptide of the  
30 present invention can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered

to a subject. A number of illustrative retroviral systems have been described (*e.g.*, U.S. Pat. No. 5,219,740; Miller and Rosman (1989) *BioTechniques* 7:980-990; Miller, A. D. (1990) *Human Gene Therapy* 1:5-14; Scarpa et al. (1991) *Virology* 180:849-852; Burns et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:8033-8037; and Boris-Lawrie and Temin  
5 (1993) *Cur. Opin. Genet. Develop.* 3:102-109.

In addition, a number of illustrative adenovirus-based systems have also been described. Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally thus minimizing the risks associated with insertional mutagenesis (Haj-Ahmad and Graham (1986) *J. Virol.* 57:267-274; Bett et al. (1993) *J.*  
10 *Virol.* 67:5911-5921; Mittereder et al. (1994) *Human Gene Therapy* 5:717-729; Seth et al. (1994) *J. Virol.* 68:933-940; Barr et al. (1994) *Gene Therapy* 1:51-58; Berkner, K. L. (1988) *BioTechniques* 6:616-629; and Rich et al. (1993) *Human Gene Therapy* 4:461-476).

Various adeno-associated virus (AAV) vector systems have also been  
15 developed for polynucleotide delivery. AAV vectors can be readily constructed using techniques well known in the art. See, *e.g.*, U.S. Pat. Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 and WO 93/03769; Lebkowski et al. (1988) *Molec. Cell. Biol.* 8:3988-3996; Vincent et al. (1990) *Vaccines* 90 (Cold Spring Harbor Laboratory Press); Carter, B. J. (1992) *Current Opinion in Biotechnology* 3:533-  
20 539; Muzyczka, N. (1992) *Current Topics in Microbiol. and Immunol.* 158:97-129; Kotin, R. M. (1994) *Human Gene Therapy* 5:793-801; Shelling and Smith (1994) *Gene Therapy* 1:165-169; and Zhou et al. (1994) *J. Exp. Med.* 179:1867-1875.

Additional viral vectors useful for delivering the polynucleotides encoding polypeptides of the present invention by gene transfer include those derived  
25 from the pox family of viruses, such as vaccinia virus and avian poxvirus. By way of example, vaccinia virus recombinants expressing the novel molecules can be constructed as follows. The DNA encoding a polypeptide is first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is  
30 then used to transfect cells which are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene

encoding the polypeptide of interest into the viral genome. The resulting TK.sup.(-) recombinant can be selected by culturing the cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

A vaccinia-based infection/transfection system can be conveniently used  
5 to provide for inducible, transient expression or coexpression of one or more polypeptides described herein in host cells of an organism. In this particular system, cells are first infected in vitro with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are  
10 transfected with the polynucleotide or polynucleotides of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA which is then translated into polypeptide by the host translational machinery. The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation  
15 products. See, *e.g.*, Elroy-Stein and Moss, Proc. Natl. Acad. Sci. USA (1990) 87:6743-6747; Fuerst et al. Proc. Natl. Acad. Sci. USA (1986) 83:8122-8126.

Alternatively, avipoxviruses, such as the fowlpox and canarypox viruses, can also be used to deliver the coding sequences of interest. Recombinant avipox viruses, expressing immunogens from mammalian pathogens, are known to confer  
20 protective immunity when administered to non-avian species. The use of an Avipox vector is particularly desirable in human and other mammalian species since members of the Avipox genus can only productively replicate in susceptible avian species and therefore are not infective in mammalian cells. Methods for producing recombinant Avipoxviruses are known in the art and employ genetic recombination, as described  
25 above with respect to the production of vaccinia viruses. See, *e.g.*, WO 91/12882; WO 89/03429; and WO 92/03545.

Any of a number of alphavirus vectors can also be used for delivery of polynucleotide compositions of the present invention, such as those vectors described in U.S. Patent Nos. 5,843,723; 6,015,686; 6,008,035 and 6,015,694. Certain vectors based  
30 on Venezuelan Equine Encephalitis (VEE) can also be used, illustrative examples of which can be found in U.S. Patent Nos. 5,505,947 and 5,643,576.

Moreover, molecular conjugate vectors, such as the adenovirus chimeric vectors described in Michael et al. *J. Biol. Chem.* (1993) 268:6866-6869 and Wagner et al. *Proc. Natl. Acad. Sci. USA* (1992) 89:6099-6103, can also be used for gene delivery under the invention.

- 5 Additional illustrative information on these and other known viral-based delivery systems can be found, for example, in Fisher-Hoch et al., *Proc. Natl. Acad. Sci. USA* 86:317-321, 1989; Flexner et al., *Ann. N.Y. Acad. Sci.* 569:86-103, 1989; Flexner et al., *Vaccine* 8:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805;
- 10 Berkner, *Biotechniques* 6:616-627, 1988; Rosenfeld et al., *Science* 252:431-434, 1991; Kolls et al., *Proc. Natl. Acad. Sci. USA* 91:215-219, 1994; Kass-Eisler et al., *Proc. Natl. Acad. Sci. USA* 90:11498-11502, 1993; Guzman et al., *Circulation* 88:2838-2848, 1993; and Guzman et al., *Cir. Res.* 73:1202-1207, 1993.

- In certain embodiments, a polynucleotide may be integrated into the
- 15 genome of a target cell. This integration may be in the specific location and orientation via homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the polynucleotide may be stably maintained in the cell as a separate, episomal segment of DNA. Such polynucleotide segments or "episomes" encode sequences sufficient to
- 20 permit maintenance and replication independent of or in synchronization with the host cell cycle. The manner in which the expression construct is delivered to a cell and where in the cell the polynucleotide remains is dependent on the type of expression construct employed.

- In another embodiment of the invention, a polynucleotide is
- 25 administered/delivered as "naked" DNA, for example as described in Ulmer et al., *Science* 259:1745-1749, 1993 and reviewed by Cohen, *Science* 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

- In still another embodiment, a composition of the present invention can
- 30 be delivered via a particle bombardment approach, many of which have been described. In one illustrative example, gas-driven particle acceleration can be achieved with

devices such as those manufactured by Powderject Pharmaceuticals PLC (Oxford, UK) and Powderject Vaccines Inc. (Madison, WI), some examples of which are described in U.S. Patent Nos. 5,846,796; 6,010,478; 5,865,796; 5,584,807; and EP Patent No. 0500 799. This approach offers a needle-free delivery approach wherein a dry powder  
5 formulation of microscopic particles, such as polynucleotide or polypeptide particles, are accelerated to high speed within a helium gas jet generated by a hand held device, propelling the particles into a target tissue of interest.

In a related embodiment, other devices and methods that may be useful for gas-driven needle-less injection of compositions of the present invention include  
10 those provided by Bioject, Inc. (Portland, OR), some examples of which are described in U.S. Patent Nos. 4,790,824; 5,064,413; 5,312,335; 5,383,851; 5,399,163; 5,520,639 and 5,993,412.

According to another embodiment, the pharmaceutical compositions described herein will comprise one or more immunostimulants in addition to the  
15 immunogenic polynucleotide, polypeptide, antibody, T-cell, TCR, and/or APC compositions of this invention. An immunostimulant refers to essentially any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. One preferred type of immunostimulant comprises an adjuvant. Many adjuvants contain a substance designed to protect the antigen from rapid  
20 catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bordetella pertussis* or *Mycobacterium tuberculosis* derived proteins. Certain adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham,  
25 Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF, interleukin-2, -7, -12, and other like growth factors, may  
30 also be used as adjuvants.

Within certain embodiments of the invention, the adjuvant composition is preferably one that induces an immune response predominantly of the Th1 type. High levels of Th1-type cytokines (e.g., IFN- $\gamma$ , TNF $\alpha$ , IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, 5 high levels of Th2-type cytokines (e.g., IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level 10 of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, *Ann. Rev. Immunol.* 7:145-173, 1989.

Certain preferred adjuvants for eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3- 15 de-O-acylated monophosphoryl lipid A, together with an aluminum salt. MPL<sup>®</sup> adjuvants are available from Corixa Corporation (Seattle, WA; *see*, for example, US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, 20 for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato et al., *Science* 273:352, 1996. Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or 25 *Chenopodium quinoa* saponins. Other preferred formulations include more than one saponin in the adjuvant combinations of the present invention, for example combinations of at least two of the following group comprising QS21, QS7, Quil A,  $\beta$ -escin, or digitonin.

Alternatively the saponin formulations may be combined with vaccine 30 vehicles composed of chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, poly-N-acetyl glucosamine-based polymer matrix,



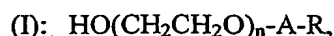
particles composed of polysaccharides or chemically modified polysaccharides, liposomes and lipid-based particles, particles composed of glycerol monoesters, etc. The saponins may also be formulated in the presence of cholesterol to form particulate structures such as liposomes or ISCOMs. Furthermore, the saponins may be formulated  
5 together with a polyoxyethylene ether or ester, in either a non-particulate solution or suspension, or in a particulate structure such as a paucilamellar liposome or ISCOM. The saponins may also be formulated with excipients such as Carbopol<sup>R</sup> to increase viscosity, or may be formulated in a dry powder form with a powder excipient such as lactose.

10 In one preferred embodiment, the adjuvant system includes the combination of a monophosphoryl lipid A and a saponin derivative, such as the combination of QS21 and 3D-MPL<sup>®</sup> adjuvant, as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and  
15 tocopherol. Another particularly preferred adjuvant formulation employing QS21, 3D-MPL<sup>®</sup> adjuvant and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Another enhanced adjuvant system involves the combination of a CpG-containing oligonucleotide and a saponin derivative particularly the combination of  
20 CpG and QS21 is disclosed in WO 00/09159. Preferably the formulation additionally comprises an oil in water emulsion and tocopherol.

Additional illustrative adjuvants for use in the pharmaceutical compositions of the invention include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series  
25 of adjuvants (e.g., SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Enhancyn<sup>®</sup>) (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties, and  
30 polyoxyethylene ether adjuvants such as those described in WO 99/52549A1.

Other preferred adjuvants include adjuvant molecules of the general formula



wherein,  $n$  is 1-50,  $A$  is a bond or  $-\text{C}(\text{O})-$ ,  $R$  is  $\text{C}_{1-50}$  alkyl or Phenyl  $\text{C}_{1-50}$  alkyl.

5 One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein  $n$  is between 1 and 50, preferably 4-24, most preferably 9; the  $R$  component is  $\text{C}_{1-50}$ , preferably  $\text{C}_4\text{-C}_{20}$  alkyl and most preferably  $\text{C}_{12}$  alkyl, and  $A$  is a bond. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably  
10 from 0.1-10%, and most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-stearyl ether, polyoxyethylene-8-stearyl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether. Polyoxyethylene ethers such as polyoxyethylene lauryl ether are described in the Merck  
15 index (12<sup>th</sup> edition: entry 7717). These adjuvant molecules are described in WO 99/52549.

The polyoxyethylene ether according to the general formula (I) above may, if desired, be combined with another adjuvant. For example, a preferred adjuvant combination is preferably with CpG as described in the pending UK patent application  
20 GB 9820956.2.

According to another embodiment of this invention, an immunogenic composition described herein is delivered to a host via antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified  
25 to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumor effects *per se* and/or to be immunologically compatible with the receiver (*i.e.*, matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, including tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic  
30 or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, *Nature* 392:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic antitumor immunity (see Timmerman and Levy, *Ann. Rev. Med.* 50:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate *in situ*, with marked cytoplasmic processes (dendrites) visible *in vitro*), their ability to take up, process and present antigens with high efficiency and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (see Zitvogel et al., *Nature Med.* 4:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, tumor-infiltrating cells, peritumoral tissues-infiltrating cells, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNF $\alpha$  to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNF $\alpha$ , CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fc $\gamma$  receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high

expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (*e.g.*, CD54 and CD11) and costimulatory molecules (*e.g.*, CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide of the invention (or portion or other variant thereof) such that the encoded polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place *ex vivo*, and a pharmaceutical composition comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo*. *In vivo* and *ex vivo* transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., *Immunology and cell Biology* 75:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the tumor polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (*e.g.*, vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (*e.g.*, a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will typically vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, mucosal, intravenous, intracranial, intraperitoneal, subcutaneous and intramuscular administration.

Carriers for use within such pharmaceutical compositions are biocompatible, and may also be biodegradable. In certain embodiments, the formulation preferably provides a relatively constant level of active component release. In other embodiments, however, a more rapid rate of release immediately upon administration may be desired. The formulation of such compositions is well within the

level of ordinary skill in the art using known techniques. Illustrative carriers useful in this regard include microparticles of poly(lactide-co-glycolide), polyacrylate, latex, starch, cellulose, dextran and the like. Other illustrative delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (*e.g.*,  
5 a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (*see e.g.*, U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of  
10 the condition to be treated or prevented.

In another illustrative embodiment, biodegradable microspheres (*e.g.*, polylactate polyglycolate) are employed as carriers for the compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268; 5,075,109; 5,928,647; 5,811,128; 5,820,883; 5,853,763;  
15 5,814,344, 5,407,609 and 5,942,252. Modified hepatitis B core protein carrier systems, such as described in WO/99 40934, and references cited therein, will also be useful for many applications. Another illustrative carrier/delivery system employs a carrier comprising particulate-protein complexes, such as those described in U.S. Patent No. 5,928,647, which are capable of inducing a class I-restricted cytotoxic T lymphocyte  
20 responses in a host.

In another illustrative embodiment, calcium phosphate core particles are employed as carriers, vaccine adjuvants, or as controlled release matrices for the compositions of this invention. Exemplary calcium phosphate particles are disclosed, for example, in published patent application No. WO/0046147.

25 The pharmaceutical compositions of the invention will often further comprise one or more buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide), solutes that  
30 render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a

recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate.

The pharmaceutical compositions described herein may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers  
5 are typically sealed in such a way to preserve the sterility and stability of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles. Alternatively, a pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

10 The development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including *e.g.*, oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation, is well known in the art, some of which are briefly discussed below for general purposes of illustration.

15 In certain applications, the pharmaceutical compositions disclosed herein may be delivered *via* oral administration to an animal. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard- or soft-shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

20 The active compounds may even be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (see, for example, Mathiowitz *et al.*, Nature 1997 Mar 27;386(6623):410-4; Hwang *et al.*, Crit Rev Ther Drug Carrier Syst 1998;15(3):243-84; U. S. Patent 5,641,515; U. S. Patent 5,580,579 and U. S. Patent  
25 5,792,451). Tablets, troches, pills, capsules and the like may also contain any of a variety of additional components, for example, a binder, such as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may  
30 be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to

materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

Typically, these formulations will contain at least about 0.1% of the active compound or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 60% or 70% or more of the weight or volume of the total formulation. Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared in such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

For oral administration the compositions of the present invention may alternatively be incorporated with one or more excipients in the form of a mouthwash, dentifrice, buccal tablet, oral spray, or sublingual orally-administered formulation. Alternatively, the active ingredient may be incorporated into an oral solution such as one containing sodium borate, glycerin and potassium bicarbonate, or dispersed in a dentifrice, or added in a therapeutically-effective amount to a composition that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants. Alternatively the compositions may be fashioned into a tablet or solution form that may be placed under the tongue or otherwise dissolved in the mouth.

In certain circumstances it will be desirable to deliver the pharmaceutical compositions disclosed herein parenterally, intravenously, intramuscularly, or even intraperitoneally. Such approaches are well known to the skilled artisan, some of which are further described, for example, in U. S. Patent 5,543,158; U. S. Patent 5,641,515 and U. S. Patent 5,399,363. In certain embodiments, solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably

mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations generally will contain a preservative to prevent the growth of microorganisms.

- 5                    Illustrative pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (for example, see U. S. Patent 5,466,468). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and  
10 storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as  
15 lecithin, by the maintenance of the required particle size in the case of dispersion and/or by the use of surfactants. The prevention of the action of microorganisms can be facilitated by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride.  
20 Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

- In one embodiment, for parenteral administration in an aqueous solution, the solution should be suitably buffered if necessary and the liquid diluent first rendered  
25 isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml  
30 of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-



1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. Moreover, for human administration, preparations will of course preferably meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

5               In another embodiment of the invention, the compositions disclosed herein may be formulated in a neutral or salt form. Illustrative pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, 10 tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount 15 as is therapeutically effective.

The carriers can further comprise any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. 20 Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human.

25               In certain embodiments, the pharmaceutical compositions may be delivered by intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering genes, nucleic acids, and peptide compositions directly to the lungs *via* nasal aerosol sprays has been described, *e.g.*, in U. S. Patent 5,756,353 and U. S. Patent 5,804,212. Likewise, the delivery of drugs using intranasal microparticle 30 resins (Takenaga *et al.*, *J Controlled Release* 1998 Mar 2;52(1-2):81-7) and lysophosphatidyl-glycerol compounds (U. S. Patent 5,725,871) are also well-known in

the pharmaceutical arts. Likewise, illustrative transmucosal drug delivery in the form of a polytetrafluoroethylene support matrix is described in U. S. Patent 5,780,045.

In certain embodiments, liposomes, nanocapsules, microparticles, lipid particles, vesicles, and the like, are used for the introduction of the compositions of the present invention into suitable host cells/organisms. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like. Alternatively, compositions of the present invention can be bound, either covalently or non-covalently, to the surface of such carrier vehicles.

10 The formation and use of liposome and liposome-like preparations as potential drug carriers is generally known to those of skill in the art (see for example, Lasic, Trends Biotechnol 1998 Jul;16(7):307-21; Takakura, Nippon Rinsho 1998 Mar;56(3):691-5; Chandran *et al.*, Indian J Exp Biol. 1997 Aug;35(8):801-9; Margalit, Crit Rev Ther Drug Carrier Syst. 1995;12(2-3):233-61; U.S. Patent 5,567,434; U.S. Patent 5,552,157; U.S. Patent 5,565,213; U.S. Patent 5,738,868 and U.S. Patent 5,795,587, each specifically incorporated herein by reference in its entirety).

Liposomes have been used successfully with a number of cell types that are normally difficult to transfect by other procedures, including T cell suspensions, primary hepatocyte cultures and PC 12 cells (Renneisen *et al.*, J Biol Chem. 1990 Sep 25;265(27):16337-42; Muller *et al.*, DNA Cell Biol. 1990 Apr;9(3):221-9). In addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, various drugs, radiotherapeutic agents, enzymes, viruses, transcription factors, allosteric effectors and the like, into a variety of cultured cell lines and animals. Furthermore, the use of liposomes does not appear to be associated with autoimmune responses or unacceptable toxicity after systemic delivery.

In certain embodiments, liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)).

30 Alternatively, in other embodiments, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the

present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (see, for example, Quintanar-Guerrero *et al.*, Drug Dev Ind Pharm. 1998 Dec;24(12):1113-28). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1  $\mu\text{m}$ ) may be designed using  
5 polymers able to be degraded *in vivo*. Such particles can be made as described, for example, by Couvreur *et al.*, Crit Rev Ther Drug Carrier Syst. 1988;5(1):1-20; zur Muhlen *et al.*, Eur J Pharm Biopharm. 1998 Mar;45(2):149-55; Zambaux *et al.* J Controlled Release. 1998 Jan 2;50(1-3):31-40; and U. S. Patent 5,145,684.

#### CANCER THERAPEUTIC METHODS

10 Immunologic approaches to cancer therapy are based on the recognition that cancer cells can often evade the body's defenses against aberrant or foreign cells and molecules, and that these defenses might be therapeutically stimulated to regain the lost ground, *e.g.* pgs. 623-648 in Klein, Immunology (Wiley-Interscience, New York, 1982). Numerous recent observations that various immune effectors can directly or  
15 indirectly inhibit growth of tumors has led to renewed interest in this approach to cancer therapy, *e.g.* Jager, et al., Oncology 2001;60(1):1-7; Renner, et al., Ann Hematol 2000 Dec;79(12):651-9.

Four-basic cell types whose function has been associated with antitumor cell immunity and the elimination of tumor cells from the body are: i) B-lymphocytes  
20 which secrete immunoglobulins into the blood plasma for identifying and labeling the nonself invader cells; ii) monocytes which secrete the complement proteins that are responsible for lysing and processing the immunoglobulin-coated target invader cells; iii) natural killer lymphocytes having two mechanisms for the destruction of tumor cells, antibody-dependent cellular cytotoxicity and natural killing; and iv) T-  
25 lymphocytes possessing antigen-specific receptors and having the capacity to recognize a tumor cell carrying complementary marker molecules (Schreiber, H., 1989, in Fundamental Immunology (ed). W. E. Paul, pp. 923-955).

Cancer immunotherapy generally focuses on inducing humoral immune responses, cellular immune responses, or both. Moreover, it is well established that  
30 induction of CD4<sup>+</sup> T helper cells is necessary in order to secondarily induce either

antibodies or cytotoxic CD8<sup>+</sup> T cells. Polypeptide antigens that are selective or ideally specific for cancer cells, particularly colon cancer cells, offer a powerful approach for inducing immune responses against colon cancer, and are an important aspect of the present invention.

5                   Therefore, in further aspects of the present invention, the pharmaceutical compositions described herein may be used to stimulate an immune response against cancer, particularly for the immunotherapy of colon cancer. Within such methods, the pharmaceutical compositions described herein are administered to a patient, typically a warm-blooded animal, preferably a human. A patient may or may not be afflicted with  
10 cancer. Pharmaceutical compositions and vaccines may be administered either prior to or following surgical removal of primary tumors and/or treatment such as administration of radiotherapy or conventional chemotherapeutic drugs. As discussed above, administration of the pharmaceutical compositions may be by any suitable method, including administration by intravenous, intraperitoneal, intramuscular,  
15 subcutaneous, intranasal, intradermal, anal, vaginal, topical and oral routes.

                  Within certain embodiments, immunotherapy may be active immunotherapy, in which treatment relies on the *in vivo* stimulation of the endogenous host immune system to react against tumors with the administration of immune response-modifying agents (such as polypeptides and polynucleotides as provided  
20 herein).

                  Within other embodiments, immunotherapy may be passive immunotherapy, in which treatment involves the delivery of agents with established tumor-immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate antitumor effects and does not necessarily depend on an intact host  
25 immune system. Examples of effector cells include T cells as discussed above, T lymphocytes (such as CD8<sup>+</sup> cytotoxic T lymphocytes and CD4<sup>+</sup> T-helper tumor-infiltrating lymphocytes), killer cells (such as Natural Killer cells and lymphokine-activated killer cells), B cells and antigen-presenting cells (such as dendritic cells and macrophages) expressing a polypeptide provided herein. T cell receptors and antibody  
30 receptors specific for the polypeptides recited herein may be cloned, expressed and transferred into other vectors or effector cells for adoptive immunotherapy. The

polypeptides provided herein may also be used to generate antibodies or anti-idiotypic antibodies (as described above and in U.S. Patent No. 4,918,164) for passive immunotherapy.

Monoclonal antibodies may be labeled with any of a variety of labels for  
5 desired selective usages in detection, diagnostic assays or therapeutic applications (as described in U.S. Patent Nos. 6,090,365; 6,015,542; 5,843,398; 5,595,721; and 4,708,930, hereby incorporated by reference in their entirety as if each was incorporated individually). In each case, the binding of the labelled monoclonal antibody to the  
10 determinant site of the antigen will signal detection or delivery of a particular therapeutic agent to the antigenic determinant on the non-normal cell. A further object of this invention is to provide the specific monoclonal antibody suitably labelled for achieving such desired selective usages thereof.

Effector cells may generally be obtained in sufficient quantities for adoptive immunotherapy by growth *in vitro*, as described herein. Culture conditions for  
15 expanding single antigen-specific effector cells to several billion in number with retention of antigen recognition *in vivo* are well known in the art. Such *in vitro* culture conditions typically use intermittent stimulation with antigen, often in the presence of cytokines (such as IL-2) and non-dividing feeder cells. As noted above, immunoreactive polypeptides as provided herein may be used to rapidly expand  
20 antigen-specific T cell cultures in order to generate a sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast and/or B cells, may be pulsed with immunoreactive polypeptides or transfected with one or more polynucleotides using standard techniques well known in the art. For example, antigen-presenting cells can be transfected with a  
25 polynucleotide having a promoter appropriate for increasing expression in a recombinant virus or other expression system. Cultured effector cells for use in therapy must be able to grow and distribute widely, and to survive long term *in vivo*. Studies have shown that cultured effector cells can be induced to grow *in vivo* and to survive long term in substantial numbers by repeated stimulation with antigen supplemented  
30 with IL-2 (*see*, for example, Cheever et al., *Immunological Reviews* 157:177, 1997).

Alternatively, a vector expressing a polypeptide recited herein may be introduced into antigen presenting cells taken from a patient and clonally propagated *ex vivo* for transplant back into the same patient. Transfected cells may be reintroduced into the patient using any means known in the art, preferably in sterile form by intravenous, intracavitary, intraperitoneal or intratumor administration.

Routes and frequency of administration of the therapeutic compositions described herein, as well as dosage, will vary from individual to individual, and may be readily established using standard techniques. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Preferably, between 1 and 10 doses may be administered over a 52 week period. Preferably, 6 doses are administered, at intervals of 1 month, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of a compound that, when administered as described above, is capable of promoting an anti-tumor immune response, and is at least 10-50% above the basal (*i.e.*, untreated) level. Such response can be monitored by measuring the anti-tumor antibodies in a patient or by vaccine-dependent generation of cytolytic effector cells capable of killing the patient's tumor cells *in vitro*. Such vaccines should also be capable of causing an immune response that leads to an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial or longer disease-free survival) in vaccinated patients as compared to non-vaccinated patients. In general, for pharmaceutical compositions and vaccines comprising one or more polypeptides, the amount of each polypeptide present in a dose ranges from about 25  $\mu$ g to 5 mg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to non-treated patients. Increases in preexisting immune responses to a tumor protein generally correlate with an improved

clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

#### CANCER DETECTION AND DIAGNOSTIC COMPOSITIONS, METHODS AND KITS

5           In general, a cancer may be detected in a patient based on the presence of one or more colon tumor proteins and/or polynucleotides encoding such proteins in a biological sample (for example, blood, sera, sputum urine and/or tumor biopsies) obtained from the patient. In other words, such proteins may be used as markers to indicate the presence or absence of a cancer such as colon cancer. In addition, such  
10 proteins may be useful for the detection of other cancers. The binding agents provided herein generally permit detection of the level of antigen that binds to the agent in the biological sample.

Polynucleotide primers and probes may be used to detect the level of mRNA encoding a tumor protein, which is also indicative of the presence or absence of  
15 a cancer. In general, a tumor sequence should be present at a level that is at least two-fold, preferably three-fold, and more preferably five-fold or higher in tumor tissue than in normal tissue of the same type from which the tumor arose. Expression levels of a particular tumor sequence in tissue types different from that in which the tumor arose are irrelevant in certain diagnostic embodiments since the presence of tumor cells can  
20 be confirmed by observation of predetermined differential expression levels, e.g., 2-fold, 5-fold, etc, in tumor tissue to expression levels in normal tissue of the same type.

Other differential expression patterns can be utilized advantageously for diagnostic purposes. For example, in one aspect of the invention, overexpression of a tumor sequence in tumor tissue and normal tissue of the same type, but not in other  
25 normal tissue types, e.g. PBMCs, can be exploited diagnostically. In this case, the presence of metastatic tumor cells, for example in a sample taken from the circulation or some other tissue site different from that in which the tumor arose, can be identified and/or confirmed by detecting expression of the tumor sequence in the sample, for example using RT-PCR analysis. In many instances, it will be desired to enrich for

tumor cells in the sample of interest, e.g., PBMCs, using cell capture or other like techniques.

There are a variety of assay formats known to those of ordinary skill in the art for using a binding agent to detect polypeptide markers in a sample. *See, e.g.,* 5 Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, the presence or absence of a cancer in a patient may be determined by (a) contacting a biological sample obtained from a patient with a binding agent; (b) detecting in the sample a level of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined cut-off value.

10 In a preferred embodiment, the assay involves the use of binding agent immobilized on a solid support to bind to and remove the polypeptide from the remainder of the sample. The bound polypeptide may then be detected using a detection reagent that contains a reporter group and specifically binds to the binding agent/polypeptide complex. Such detection reagents may comprise, for example, a 15 binding agent that specifically binds to the polypeptide or an antibody or other agent that specifically binds to the binding agent, such as an anti-immunoglobulin, protein G, protein A or a lectin. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding agent after incubation of the binding agent with the sample. The extent to 20 which components of the sample inhibit the binding of the labeled polypeptide to the binding agent is indicative of the reactivity of the sample with the immobilized binding agent. Suitable polypeptides for use within such assays include full length colon tumor proteins and polypeptide portions thereof to which the binding agent binds, as described above.

25 The solid support may be any material known to those of ordinary skill in the art to which the tumor protein may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a 30 magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The binding agent may be immobilized on the solid support



using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the agent and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the binding agent, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and about 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about 10  $\mu$ g, and preferably about 100 ng to about 1  $\mu$ g, is sufficient to immobilize an adequate amount of binding agent.

Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (*see, e.g.,* Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that polypeptides within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide-antibody complexes and a detection reagent (preferably a second antibody capable of binding to a different site on the polypeptide) containing a reporter group is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific reporter group.

More specifically, once the antibody is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO). The  
5 immobilized antibody is then incubated with the sample, and polypeptide is allowed to bind to the antibody. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.*, incubation time) is a period of time that is sufficient to detect the presence of polypeptide within a sample obtained from an individual with colon cancer at least  
10 about 95% of that achieved at equilibrium between bound and unbound polypeptide. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

15 Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. The second antibody, which contains a reporter group, may then be added to the solid support. Preferred reporter groups include those groups recited above.

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound polypeptide.  
20 An appropriate amount of time may generally be determined by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter  
25 group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate  
30 (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of a cancer, such as colon cancer, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value for the detection of a cancer is the average  
5 mean signal obtained when the immobilized antibody is incubated with samples from patients without the cancer. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for the cancer. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical*  
10 *Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, p. 106-7. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the  
15 value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off  
20 value determined by this method is considered positive for a cancer.

In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the binding agent is immobilized on a membrane, such as nitrocellulose. In the flow-through test, polypeptides within the sample bind to the immobilized binding agent as the sample passes through the membrane. A second,  
25 labeled binding agent then binds to the binding agent-polypeptide complex as a solution containing the second binding agent flows through the membrane. The detection of bound second binding agent may then be performed as described above. In the strip test format, one end of the membrane to which binding agent is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a  
30 region containing second binding agent and to the area of immobilized binding agent. Concentration of second binding agent at the area of immobilized antibody indicates the

presence of a cancer. Typically, the concentration of second binding agent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of binding agent immobilized on the membrane is selected to generate a visually discernible pattern when the  
5 biological sample contains a level of polypeptide that would be sufficient to generate a positive signal in the two-antibody sandwich assay, in the format discussed above. Preferred binding agents for use in such assays are antibodies and antigen-binding fragments thereof. Preferably, the amount of antibody immobilized on the membrane ranges from about 25 ng to about 1  $\mu$ g, and more preferably from about 50 ng to about  
10 500 ng. Such tests can typically be performed with a very small amount of biological sample.

Of course, numerous other assay protocols exist that are suitable for use with the tumor proteins or binding agents of the present invention. The above descriptions are intended to be exemplary only. For example, it will be apparent to  
15 those of ordinary skill in the art that the above protocols may be readily modified to use tumor polypeptides to detect antibodies that bind to such polypeptides in a biological sample. The detection of such tumor protein specific antibodies may correlate with the presence of a cancer.

A cancer may also, or alternatively, be detected based on the presence of  
20 T cells that specifically react with a tumor protein in a biological sample. Within certain methods, a biological sample comprising CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells isolated from a patient is incubated with a tumor polypeptide, a polynucleotide encoding such a polypeptide and/or an APC that expresses at least an immunogenic portion of such a polypeptide, and the presence or absence of specific activation of the T cells is detected.  
25 Suitable biological samples include, but are not limited to, isolated T cells. For example, T cells may be isolated from a patient by routine techniques (such as by Ficoll/Hypaque density gradient centrifugation of peripheral blood lymphocytes). T cells may be incubated *in vitro* for 2-9 days (typically 4 days) at 37°C with polypeptide (e.g., 5 - 25  $\mu$ g/ml). It may be desirable to incubate another aliquot of a T cell sample  
30 in the absence of tumor polypeptide to serve as a control. For CD4<sup>+</sup> T cells, activation is preferably detected by evaluating proliferation of the T cells. For CD8<sup>+</sup> T cells,

activation is preferably detected by evaluating cytolytic activity. A level of proliferation that is at least two fold greater and/or a level of cytolytic activity that is at least 20% greater than in disease-free patients indicates the presence of a cancer in the patient.

5 As noted above, a cancer may also, or alternatively, be detected based on the level of mRNA encoding a tumor protein in a biological sample. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify a portion of a tumor cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for (*i.e.*,  
10 hybridizes to) a polynucleotide encoding the tumor protein. The amplified cDNA is then separated and detected using techniques well known in the art, such as gel electrophoresis.

Similarly, oligonucleotide probes that specifically hybridize to a polynucleotide encoding a tumor protein may be used in a hybridization assay to detect  
15 the presence of polynucleotide encoding the tumor protein in a biological sample.

To permit hybridization under assay conditions, oligonucleotide primers and probes should comprise an oligonucleotide sequence that has at least about 60%, preferably at least about 75% and more preferably at least about 90%, identity to a portion of a polynucleotide encoding a tumor protein of the invention that is at least 10  
20 nucleotides, and preferably at least 20 nucleotides, in length. Preferably, oligonucleotide primers and/or probes hybridize to a polynucleotide encoding a polypeptide described herein under moderately stringent conditions, as defined above. Oligonucleotide primers and/or probes which may be usefully employed in the diagnostic methods described herein preferably are at least 10-40 nucleotides in length.  
25 In a preferred embodiment, the oligonucleotide primers comprise at least 10 contiguous nucleotides, more preferably at least 15 contiguous nucleotides, of a DNA molecule having a sequence as disclosed herein. Techniques for both PCR based assays and hybridization assays are well known in the art (*see*, for example, Mullis et al., *Cold Spring Harbor Symp. Quant. Biol.*, 51:263, 1987; Erlich ed., *PCR Technology*, Stockton  
30 Press, NY, 1989).

One preferred assay employs RT-PCR, in which PCR is applied in conjunction with reverse transcription. Typically, RNA is extracted from a biological sample, such as biopsy tissue, and is reverse transcribed to produce cDNA molecules. PCR amplification using at least one specific primer generates a cDNA molecule, which may be separated and visualized using, for example, gel electrophoresis. Amplification may be performed on biological samples taken from a test patient and from an individual who is not afflicted with a cancer. The amplification reaction may be performed on several dilutions of cDNA spanning two orders of magnitude. A two-fold or greater increase in expression in several dilutions of the test patient sample as compared to the same dilutions of the non-cancerous sample is typically considered positive.

In another aspect of the present invention, cell capture technologies may be used in conjunction, with, for example, real-time PCR to provide a more sensitive tool for detection of metastatic cells expressing colon tumor antigens. Detection of colon cancer cells in biological samples, e.g., bone marrow samples, peripheral blood, and small needle aspiration samples is desirable for diagnosis and prognosis in colon cancer patients.

Immunomagnetic beads coated with specific monoclonal antibodies to surface cell markers, or tetrameric antibody complexes, may be used to first enrich or positively select cancer cells in a sample. Various commercially available kits may be used, including Dynabeads® Epithelial Enrich (DynaL Biotech, Oslo, Norway), StemSep™ (StemCell Technologies, Inc., Vancouver, BC), and RosetteSep (StemCell Technologies). A skilled artisan will recognize that other methodologies and kits may also be used to enrich or positively select desired cell populations. Dynabeads® Epithelial Enrich contains magnetic beads coated with mAbs specific for two glycoprotein membrane antigens expressed on normal and neoplastic epithelial tissues. The coated beads may be added to a sample and the sample then applied to a magnet, thereby capturing the cells bound to the beads. The unwanted cells are washed away and the magnetically isolated cells eluted from the beads and used in further analyses.

RosetteSep can be used to enrich cells directly from a blood sample and consists of a cocktail of tetrameric antibodies that targets a variety of unwanted cells

and crosslinks them to glycophorin A on red blood cells (RBC) present in the sample, forming rosettes. When centrifuged over Ficoll, targeted cells pellet along with the free RBC. The combination of antibodies in the depletion cocktail determines which cells will be removed and consequently which cells will be recovered. Antibodies that are  
5 available include, but are not limited to: CD2, CD3, CD4, CD5, CD8, CD10, CD11b, CD14, CD15, CD16, CD19, CD20, CD24, CD25, CD29, CD33, CD34, CD36, CD38, CD41, CD45, CD45RA, CD45RO, CD56, CD66B, CD66e, HLA-DR, IgE, and TCR $\alpha\beta$ .

Additionally, it is contemplated in the present invention that mAbs  
10 specific for colon tumor antigens can be generated and used in a similar manner. For example, mAbs that bind to tumor-specific cell surface antigens may be conjugated to magnetic beads, or formulated in a tetrameric antibody complex, and used to enrich or positively select metastatic colon tumor cells from a sample. Once a sample is enriched or positively selected, cells may be lysed and RNA isolated. RNA may then be  
15 subjected to RT-PCR analysis using colon tumor-specific primers in a real-time PCR assay as described herein. One skilled in the art will recognize that enriched or selected populations of cells may be analyzed by other methods (*e.g. in situ* hybridization or flow cytometry).

In another embodiment, the compositions described herein may be used  
20 as markers for the progression of cancer. In this embodiment, assays as described above for the diagnosis of a cancer may be performed over time, and the change in the level of reactive polypeptide(s) or polynucleotide(s) evaluated. For example, the assays may be performed every 24-72 hours for a period of 6 months to 1 year, and thereafter performed as needed. In general, a cancer is progressing in those patients in whom the  
25 level of polypeptide or polynucleotide detected increases over time. In contrast, the cancer is not progressing when the level of reactive polypeptide or polynucleotide either remains constant or decreases with time.

Certain *in vivo* diagnostic assays may be performed directly on a tumor. One such assay involves contacting tumor cells with a binding agent. The bound  
30 binding agent may then be detected directly or indirectly via a reporter group. Such

binding agents may also be used in histological applications. Alternatively, polynucleotide probes may be used within such applications.

As noted above, to improve sensitivity, multiple tumor protein markers may be assayed within a given sample. It will be apparent that binding agents specific  
5 for different proteins provided herein may be combined within a single assay. Further, multiple primers or probes may be used concurrently. The selection of tumor protein markers may be based on routine experiments to determine combinations that results in optimal sensitivity. In addition, or alternatively, assays for tumor proteins provided herein may be combined with assays for other known tumor antigens.

10 The present invention further provides kits for use within any of the above diagnostic methods. Such kits typically comprise two or more components necessary for performing a diagnostic assay. Components may be compounds, reagents, containers and/or equipment. For example, one container within a kit may contain a monoclonal antibody or fragment thereof that specifically binds to a tumor  
15 protein. Such antibodies or fragments may be provided attached to a support material, as described above. One or more additional containers may enclose elements, such as reagents or buffers, to be used in the assay. Such kits may also, or alternatively, contain a detection reagent as described above that contains a reporter group suitable for direct or indirect detection of antibody binding.

20 Alternatively, a kit may be designed to detect the level of mRNA encoding a tumor protein in a biological sample. Such kits generally comprise at least one oligonucleotide probe or primer, as described above, that hybridizes to a polynucleotide encoding a tumor protein. Such an oligonucleotide may be used, for example, within a PCR or hybridization assay. Additional components that may be  
25 present within such kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate the detection of a polynucleotide encoding a tumor protein.

The following Examples are offered by way of illustration and not by way of limitation.



## EXAMPLES

### EXAMPLE 1

#### IDENTIFICATION OF COLON TUMOR PROTEIN CDNAS

5           This Example illustrates the identification of cDNA molecules encoding colon tumor proteins using PCR-based cDNA subtraction methodology.

          A modification of the Clontech (Palo Alto, CA) PCR-Select™ cDNA subtraction methodology was employed to obtain cDNA populations enriched in cDNAs derived from transcripts that are differentially expressed in colon tumor  
10   samples. By this methodology, mRNA populations were isolated from colon tumor and metastatic tumor samples ("tester" mRNA) as well as from normal tissues, such as brain, pancreas, bone marrow, liver, heart, lung, stomach and small intestine ("driver" mRNA). From the tester and driver mRNA populations, cDNA was synthesized by standard methodology. *See, e.g., Ausubel, F.M. et al., Short Protocols in Molecular*  
15 *Biology* (4<sup>th</sup> ed., John Wiley and Sons, Inc., 1999).

          The subtraction steps were performed using a PCR-based protocol that was modified to generate fragments larger than would be derived by the Clontech methodology. By this modified protocol, the tester and driver cDNAs were separately digested with five restriction endonucleases (Mlu I, Msc I, Pvu II, Sal I and Stu I) each  
20   of which recognize a unique 6-base pair nucleotide sequence. This digestion resulted in an average cDNA size of 600 bp, rather than the average size of 300 bp that results from digestion with Rsa I according to the Clontech methodology. This modification did not affect the ultimate subtraction efficiency.

          Following the restriction digestion, adapter oligonucleotides having  
25   unique nucleotide sequences were ligated onto the 5' ends of the tester cDNAs; adapter oligonucleotides were not ligated onto the driver cDNAs. The tester and driver cDNAs were subsequently hybridized one to the other using an excess of driver cDNA. This hybridization step resulted in populations of (a) unhybridized tester cDNAs, (b) tester cDNAs hybridized to other tester cDNAs, (c) tester cDNAs hybridized to driver  
30   cDNAs, (d) unhybridized driver cDNAs and (e) driver cDNAs hybridized to driver cDNAs.

Tester cDNAs hybridized to other tester cDNAs were selectively amplified by a polymerase chain reaction (PCR) employing primers complementary to the ligated adapters. Because only tester cDNAs were ligated to adapter sequences, neither unhybridized tester or driver cDNAs, tester cDNAs hybridized to driver cDNAs nor driver cDNAs hybridized to driver cDNAs were amplified using adapter specific oligonucleotides. The PCR amplified tester cDNAs were cloned into the pCR2.1 plasmid vector (Invitrogen; Carlsbad, CA) to create a libraries enriched in differentially expressed colon tumor antigen and colon metastatic tumor antigen specific cDNAs.

Three thousand clones from the pCR2.1 tumor antigen cDNA libraries were randomly selected and used to obtain clones for microarray analysis (performed by Rosetta; Seattle, WA) and nucleotide sequencing. The cDNA insert from each pCR2.1 clone was PCR amplified as follows. Briefly, 0.5  $\mu$ l of glycerol stock solution was added to 99.5  $\mu$ l of PCR mix containing 80  $\mu$ l H<sub>2</sub>O, 10  $\mu$ l 10X PCR Buffer, 6  $\mu$ l MgCl<sub>2</sub>, 1  $\mu$ l 10 mM dNTPs, 1  $\mu$ l 100 mM M13 forward primer (CACGACGTTGTAAAACGACGG), 1  $\mu$ l 100 mM M13 reverse primer (CACAGGAAACAGCTATGACC), and 0.5  $\mu$ l 5 u/ml Taq DNA polymerase. The M13 forward and reverse primers used herein were obtained from Operon Technologies (Alameda, CA). The PCR amplification was performed for thirty cycles under the following conditions: 95°C for 5 minutes, 92°C for 30 seconds, 57°C for 40 seconds, 75°C for 2 minutes and 75°C for 5 minutes.

mRNA expression levels for representative clones were determined using microarray technology in colon tumor tissues (n=25), normal colon tissues (n=6), kidney, lung, liver, brain, heart, esophagus, small intestine, stomach, pancreas, adrenal gland, salivary gland, resting PBMC, activated PBMC, bone marrow, dendritic cells, spinal cord, blood vessels, skeletal muscle, skin, breast and fetal tissues. An exemplary methodology for performing the microarray analysis is described in Schena *et al.*, *Science* 270:467-470. The number of tissue samples tested in each case was one (n=1), except where specifically noted above; additionally, all the above-mentioned tissues were derived from humans.

The PCR amplification products were dotted onto slides in an array format, with each product occupying a unique location in the array. mRNA was

extracted from the tissue sample to be tested, and fluorescent-labeled cDNA probes were generated by reverse transcription, according to standard methodology, in the presence of fluorescent nucleotides  $\psi 5$  and  $\psi 3$ . See, e.g., Ausubel, et al., *supra* for exemplary reaction conditions for performing the reverse transcription reaction;  $\psi 5$  and  $\psi 3$  fluorescent labeled nucleotides may be obtained, e.g., from Amersham Pharmacia (Uppsala, Sweden) or NEN® Life Science Products, Inc. (Boston, MA). The microarrays were probed with the fluorescent-labeled cDNAs, the slides were scanned and fluorescence intensity was measured. Genetic MicroSystems instrumentation for preparing the cDNA microarrays and for measuring fluorescence intensity is available from Affymetrix (Santa Clara, CA).

An elevated fluorescence intensity in a microarray sector probed with cDNA probes obtained from a colon tumor or colon metastatic tumor tissue as compared to the fluorescence intensity in the same sector probed with cDNA probes obtained from a normal tissue indicates a tumor antigen gene that is differentially expressed in colon tumor or colon metastatic tumor tissue.

Clones disclosed herein as SEQ ID NOs: 1-234 and described in Tables 2-4 were identified from the PCR subtracted differential colon tumor and colon metastatic tumor cDNA libraries by the microarray based methodology. Of these 234 clones, those corresponding to SEQ ID NOs: 1, 6, 18-20, 27, 30, 37, 40, 57, 65, 81, 82, 86, 88, 91, 95, 96, 106, 107, 117, 121, 123, 126, 130, 148, 150, 152, 155, 157, 159, 161, 174, 175, 180, 182, 187, 190, 191, 192, 203, 204 and 209 showed no significant similarity to known sequences in Genbank.

TABLE 2

CDNA SEQUENCES SHOWING NO SIGNIFICANT SIMILARITY TO SEQUENCE IN GENBANK

Clone	SEQ ID NO.	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54172	1	Parathyroid/breast	p0022r16c12	R0085 H6	3.24	0.276	0.085	5G12
54034	6	Ovarian	p0018r08c10	R0067 H5	2.24	0.179	0.08	4D6
53949	18	Colon/pancreatic islet	p0016r15c12	R0061 F6	2.32	0.145	0.062	3E 5
53898	19	Colon/Gastric	p0016r01c14	R0058 B7	4.43	0.423	0.095	3A2
54069	20	Prostate/colon	p0019r03c02	R0070 F1	2.5	0.136	0.054	4G5
54089	27	Colon/HCC cell line	p0019r14c18	R0073 D9	2.97	0.096	0.032	5A1
54181	30	Br/Li/Ut/Pr	p0023r09c19	R0088 A10	2.85	0.264	0.092	5H9
54147	37	Colon only	p0021r12c01	R0080 G1	2.05	0.132	0.064	5E 11
54039	40	Ovary	p0018r09c06	R0068 B3	2.03	0.185	0.091	4D11
54059	57	Novel	p0018r13c20	R0069 B10	2.02	0.089	0.044	4F7
54141	65	HCC cell line/colon/testis	p0021r07c03	R0079 E2	2.35	0.106	0.045	5E 5
54120	81	Novel	p0020r11c07	R0076 E4	2.02	0.087	0.043	5C8
54145	82	Ut/Plac/Br/Pr	p0021r11c01	R0080 E1	2.5	0.147	0.059	5E 9
54152	86	Ut/Lu/Co/Pancreatic islet	p0021r14c23	R0081 C12	2.14	0.141	0.066	5F4
54146	88	Br/Co/melanocyte	p0021r11c19	R0080 E10	2.07	0.097	0.047	5E 10
54020	91	Fetal liver/heart	p0017r16c12	R0065 H6	2.18	0.133	0.061	4C4
54161	95	Fetal liver spleen	p0022r05c16	R0083 B8	2.07	0.083	0.04	5G1
54162	96	Lot EST	p0022r05c22	R0083 B11	3.74	0.205	0.055	5G2
54098	106	Lot EST	p0020r02c05	R0074 C3	2.06	0.064	0.031	5A10
54173	107	Co/Pan/Kid/Liver	p0022r16c23	R0085 G12	2.62	0.14	0.053	5H1

Clone	SEQ ID NO.	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54183	117	Co/Bm/Ut/Lu	p0023r10c20	R0088 D10	2.8	0.092	0.033	5H11
53918	121	Infant brain/breast	p0016r07c15	R0059 E8	2.06	0.104	0.051	3B10
53910	123	Co/Ut	p0016r05c11	R0059 A6	2.01	0.098	0.049	3B2
53917	126	Infant brain/gall bladder	p0016r07c02	R0059 F1	2	0.102	0.051	3B9
53999	130	Kid/Thymus/Co	p0017r12c08	R0064 H4	2.75	0.269	0.098	4A7
54074	148	Pr	p0019r04c04	R0070 H2	2	0.198	0.099	4G10
53961	150	Novel	p0017r03c06	R0062 F3	3.45	0.069	0.02	3F5
53933	152	Lot EST	p0016r10c21	R0060 C11	2.64	0.14	0.053	3D1
53924	155	Novel	p0016r08c11	R0059 G6	3.14	0.144	0.046	3C4
54068	157	Lot EST	p0019r01c12	R0070 B6	2.01	0.182	0.091	4G4
53959	159	Germinal center B cell	p0017r03c01	R0062 E1	2.01	0.042	0.021	3F3
53931	161	Pr/Lu	p0016r10c17	R0060 C9	2.41	0.152	0.063	3C11
54091	174	Kid/Stomach	p0019r15c06	R0073 F3	2.1	0.076	0.036	5A3
54013	175	Fetal tissues/testis	p0017r15c03	R0065 E2	2.32	0.183	0.079	4B9
53963	180	Lot EST	p0017r03c12	R0062 F6	2.59	0.256	0.099	3F7
54067	182	Lot EST	p0018r16c20	R0069 H10	4.8	0.347	0.072	4G3
53966	187	Infant brain	p0017r04c07	R0062 G4	2.08	0.119	0.057	3F10
54094	190	Co/Fetal retina	p0019r16c01	R0073 G1	2.11	0.149	0.071	5A6
53977	191	1887043	p0017r05c12	R0063 B6	2.35	0.164	0.07	3G9
54123	192	Infant brain/multiple scler	p0020r15c04	R0077 F2	2.01	0.091	0.045	5C11
54016	203	Novel	p0017r15c16	R0065 F8	2.04	0.113	0.055	4B12
54018	204	Br/Co	p0017r15c23	R0065 E12	3.48	0.203	0.058	4C2
53988	209	Kid/Co/Fetal brain	p0017r08c20	R0063 H10	2.88	0.117	0.041	3H8

**TABLE 3**

SEQUENCES WITH SOME DEGREE OF SIMILARITY TO SEQUENCES IN GENBANK WITH NO KNOWN FUNCTION

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54104	2	PAC 75N13 on chromosome Xq21.1	Colon only	p0020r03c18	R0074 F9	2.15	0.098	0.045	5B4
54149	5	cDNA FLJ10461 fis, clone NT2RP10014 82	Ovarian	p0021r13c12	R0081 B6	2.5	0.068	0.027	5F1
53948	8	12p12 BAC RPC111- 267123	Testis/colon/liver	p0016r15c11	R0061 E6	2.05	0.147	0.072	3E 4
54026	9	Clone 164F3 on chromosome Xq21.33-23	Fetal liver/lung/colon	p0018r04c10	R0066 H5	2	0.125	0.062	4C10
54174	17	PAC clone RP1-170O19 from 7p15- p21	Colon only	p0023r03c09	R0086 E5	2.63	0.221	0.084	5H2

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54048	21	cDNA FLJ20676 fis, clone KAIA4294	Pancreatic islet/prostate	p0018r11c17	R0068 E9	5.15	0.315	0.061	4E 8
54031	22	Chromosome 17, clone hRPC.1171_I 10	Co/Pr/Ov/Ut	p0018r07c23	R0067 E12	4.66	0.454	0.098	4D3
54079	31	PAC 75N13 on chromosome Xq21.1	Co/Gas	p0019r06c18	R0071 D9	3.04	0.199	0.066	4H3
54160	33	Clone 146H21 on chromosome Xq22	Colon only	p0022r05c08	R0083 B4	3.7	0.215	0.058	5F12
54078	35	PAC 75N13 on chromosome Xq21.1	Colon only	p0019r06c09	R0071 C5	2.79	0.145	0.052	4H2
54037	41	Constitutive fragile region FRA3B sequence 90%	Pancreatic islet/colon	p0018r08c24	R0067 H12	2.37	0.128	0.054	4D9

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54052	51	cDNA FLJ10610 fis, clone NT2RP20052 93	Novel	p0018r12c21	R0068 G11	2.36	0.072	0.031	4E 12
54124	63	Clone RP1- 39G22 on chromosome 1p32.1-34.3	Kid/Ut/Infant brain	p0020r16c10	R0077 H5	2.07	0.149	0.072	5C12
54065	69	cDNA FLJ10969 fis, clone PLACE10009 09	Kid/Ut	p0018r15c19	R0069 E10	2.36	0.193	0.082	4G1
54060	70	BAC clone 215O12	Pancreatic islet	p0018r14c16	R0069 D8	2.15	0.099	0.046	4F8
54136	78	KIAA1077 protein	Bt/Pr/Ut	p0021r04c24	R0078 H12	2.27	0.112	0.049	5D12
54140	80	PAC 454G6 on chromosome 1q24	Pan/HeLa cell/Ut	p0021r06c08	R0079 D4	2.17	0.062	0.029	5E 4
54117	83	KIAA0152	Ut/Co/Br/Lu	p0020r10c13	R0076 C7	2.02	0.063	0.031	5C5
54159	90	cDNA DKFZp586O 0118	Lot	p0022r04c08	R0082 H4	2.64	0.159	0.06	5F11



Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54030	94	CGI-151/KIAA0992 protein	Endothelial cell/Sk Musc	p0018r06c22	R0067 D11	2.02	0.154	0.076	4D2
54133	101	cDNA DKFZp586I2022	Lu/Co/Ut	p0021r04c02	R0078 H1	2.63	0.136	0.052	5D9
54131	104	cDNA FLJ10549 fs, clone NT2RP2001976	Uv/GC/Pr	p0021r03c08	R0078 F4	2.03	0.083	0.041	5D7
54122	105	cDNA DKFZp434C0523	Embryo/fetal brain	p0020r12c04	R0076 H2	2.36	0.224	0.095	5C10
54179	110	cDNA FLJ10610 fs, clone NT2RP2005293	Thymus/fetal heart	p0023r08c18	R0087 H9	2.13	0.089	0.042	5H7
54027	116	cDNA FLJ10884 fs, clone NT2RP4001950	GC/testis	p0018r05c06	R0067 B3	2.15	0.181	0.084	4C11
54106	119	KIAA1289	Fetal tissue/melanocyte	p0020r04c19	R0074 G10	2.09	0.104	0.05	5B6

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53904	122	Chromosome 17, clone hRPK.692_E18	Co/fetal/placenta	p0016r03c15	R0058 E8	4.59	0.445	0.097	3A8
53903	124	cDNA FLJ10823 fis, clone NT2RP4001080	Colon only	p0016r03c12	R0058 F6	2.08	0.111	0.053	3A7
53928	133	citb_338_f_2, complete sequence	Ut/infant brain	p0016r09c19	R0060 A10	3.14	0.166	0.053	3C8
53930	139	Chromosome 19	6882084/6893421	p0016r10c04	R0060 D2	2.35	0.127	0.054	3C10
54005	143	Chromosome 5 clone CTC-436P18	GCB/infant brain	p0017r12c22	R0064 H11	2.07	0.132	0.064	4B1
54083	146	12q24 PAC RPC11-261P5	Novel	p0019r08c18	R0071 H9	2.12	0.057	0.027	4H7
54105	149	Clone RP4-621F18 on chromosome 1p11.4-21.3	Total fetus/fetal liver	p0020r04c18	R0074 H9	2.46	0.095	0.039	5B5
53906	154	cDNA FLJ10679 fis, clone NT2RP2006565	Lot EST	p0016r03c24	R0058 F12	2.04	0.13	0.064	3A10

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53942	160	KIAA1050	Fetus/fetal lung	p0016r14c05	R0061 C3	2.02	0.067	0.033	3D10
53935	162	cDNA FLJ11127 fis, clone PLACE10062 25	Co/Pan/Ov/Ut	p0016r11c08	R0060 F4	2.77	0.19	0.069	3D3
54000	165	KIAA0965	Fetus/Co/Ut	p0017r12c09	R0064 G5	2.12	0.149	0.07	4A8
53953	169	cDNA DKFZp586H 0519	Ovary/fetal brain	p0016r15c24	R0061 F12	2.49	0.141	0.057	3E9
53945	173	cDNA FLJ20127 fis, clone COL06176	Novel	p0016r14c20	R0061 D10	2.21	0.108	0.049	3E1
53987	178	Clone RP1- 155G6 on chromosome 20	HeLa/placenta/testis	p0017r08c16	R0063 H8	2.05	0.159	0.078	3H7
54057	183	PAC RPCI-1 133G21 map 21q11.1 region D21S190	Novel	p0018r13c11	R0069 A6	2.11	0.091	0.043	4F5
53960	193	BAC clone RG083M05 from 7q21- 7q22	Subtracted Hippocampus	p0017r03c02	R0062 F1	2.48	0.07	0.028	3F4

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53976	194	Human STS WI-14644		p0017r05c09	R0063 A5	2.53	0.243	0.096	3G8
54081	199	PAC RPCI-1 133G21 map 21q11.1 region D21S190	Colon only	p0019r07c10	R0071 F5	4.66	0.225	0.048	4H5
54082	200	PAC clone RP5-118517 from 7q11.23-q21	GCB/total fetus	p0019r07c16	R0071 F8	2.38	0.105	0.044	4H6
53992	202	cDNA FLJ20673 fis, clone KAIA4464	Kid/GCB/Co	p0017r11c08	R0064 F4	2.03	0.128	0.063	3H12
53973	206	KIAA0715	Colon/Brain	p0017r04c24	R0062 H12	4.39	0.196	0.045	3G5
53982	208	KIAA1225	Lym/Co	p0017r06c24	R0063 D12	2.22	0.107	0.048	3H2
53991	211	cDNA FLJ20171 fis, clone COL09761	Lu/Ut/Ct	p0017r10c21	R0064 C11	2.81	0.062	0.022	3H11

TABLE 4

CDNA SEQUENCES WITH SOME DEGREE OF SIMILARITY TO KNOWN SEQUENCES IN GENBANK

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53978	3	Glutamine:fructose-6-phosphate amidotransferase		p0017r05c14	R0063 B7	3.24	0.182	0.056	3G10
54184	4	Colon Kruppel-like factor		p0023r10c22	R0088 D11	3.55	0.222	0.062	5H12
54085	7	Human beta 2 gene		p0019r11c24	R0072 F12	2.08	0.184	0.089	4H9
53907	10	Lysyl hydroxylase isoform 2		p0016r04c04	R0058 H2	2.25	0.218	0.097	3A11
54066	11	Mucin 11		p0018r15c23	R0069 E12	3.87	0.222	0.057	4G2
54017	12	Mucin 11		p0017r15c20	R0065 F10	5.21	0.241	0.046	4C1
54006	13	Mucin 11		p0017r13c10	R0065 B5	3.97	0.246	0.062	4B2
53962	14	Epiregulin (EGF family)		p0017r03c09	R0062 E5	2.61	0.083	0.032	3F6
54028	15	Mucin 12		p0018r05c15	R0067 A8	2.14	0.068	0.032	4C12
54166	16	E1A enhancer binding protein		p0022r10c04	R0084 D2	2.5	0.226	0.09	5G6

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54154	23	Alpha topoisomerase truncated form		p0021r15c12	R0081 F6	3.22	0.315	0.098	5F6
54009	24	Cytokeratin 20		p0017r14c11	R0065 C6	4.07	0.185	0.045	4B5
54070	25	Erythroblastosis virus oncogene homolog 2		p0019r03c03	R0070 E2	2.05	0.172	0.084	4G6
53998	26	Polyadenylate binding protein II		p0017r12c07	R0064 G4	3.73	0.368	0.099	4A6
54182	28	Transforming growth factor-beta induced gene product		p0023r10c07	R0088 C4	3.14	0.21	0.067	5H10
53989	29	GDP-mannose 4,6 dehydratase		p0017r08c24	R0063 H12	3.77	0.259	0.069	3H9
54114	32	Mus fork head transcription factor gene 92%	Kid/Co/Lu/UtPr	p0020r09c13	R0076 A7	3.39	0.185	0.055	5C2

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54168	34	Glutamine:fructose-6-phosphate amidotransferase		p0022r15c16	R0085 F8	2.4	0.224	0.093	5G8
53900	36	Intestinal peptide-associated transporter HPT-1		p0016r03c01	R0058 E1	2.11	0.114	0.054	3A4
54033	38	Human proteinase activated receptor-2		p0018r08c07	R0067 G4	2.89	0.143	0.049	4D5
54022	39	GalNAc-T3 gene		p0017r16c21	R0065 G11	2.54	0.193	0.076	4C6
54129	42	CD24 signal transducer gene		p0021r02c15	R0078 C8	2.5	0.239	0.096	5D5
54054	43	Human c-myc gene		p0018r13c02	R0069 B1	3.15	0.282	0.089	4F2
54055	44	Pyroline-5-carboxylate synthase long form		p0018r13c03	R0069 A2	2.01	0.116	0.058	4F3
54046	45	Human zinc finger protein ZNF139		p0018r11c11	R0068 E6	2.39	0.179	0.075	4E 6

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54047	46	Gene for membrane cofactor protein		p0018r11c16	R0068 F8	3.09	0.196	0.063	4E 7
54040	47	Colon Kruppel-like factor		p0018r09c08	R0068 B4	5.44	0.377	0.069	4D12
54035	48	Human capping protein alpha subunit isoform 1		p0018r08c16	R0067 H8	2.17	0.157	0.072	4D7
54130	49	Ig lambda-chain		p0021r02c19	R0078 C10	2.41	0.076	0.032	5D6
54045	50	Protein tyrosine kinase	Placenta/Liver/testis	p0018r10c22	R0068 D11	2.15	0.148	0.069	4E 5
54050	52	Human microtubule-associated protein 7		p0018r11c24	R0068 F12	2.51	0.171	0.068	4E 10
54051	53	Human retinoblastoma susceptibility protein <sup>a</sup>		p0018r12c20	R0068 H10	2.02	0.172	0.085	4E 11
54178	54	Human reticulocalbin		p0023r06c09	R0087 C5	2.02	0.127	0.063	5H6



Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54148	55	Translation initiation factor eIF3 p36 subunit		p0021r13c01	R0081 A1	2.67	0.18	0.067	5E 12
54058	56	Human apurinic/apyrimidinic endonuclease		p0018r13c12	R0069 B6	2.31	0.105	0.045	4F6
54126	58	Human integral transmembrane protein 1		p0021r01c05	R0078 A3	2.31	0.117	0.051	5D2
54127	59	Human serine kinase		p0021r01c15	R0078 A8	2.31	0.171	0.074	5D3
54049	60	Human CGI-44 protein		p0018r11c18	R0068 F9	2.24	0.191	0.085	4E 9
54056	61	HADH/NADPH thyroid oxidase p138-tox protein		p0018r13c05	R0069 A3	2.41	0.149	0.062	4F4
54064	62	Human peptide transporter (TAP1) protein		p0018r15c13	R0069 E7	2.96	0.104	0.035	4F12

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54063	64	Transforming growth factor-beta induced gene product		p0018r15c10	R0069 F5	3.89	0.298	0.077	4F11
54119	66	Cytokeratin 8		p0020r11c02	R0076 F1	5.56	0.193	0.035	5C7
54111	67	Human coat protein gamma-cop		p0020r07c24	R0075 F12	2.05	0.076	0.037	5B11
54121	68	Bumetanide-sensitive Na-K-Cl cotransporter		p0020r11c20	R0076 F10	3.76	0.358	0.095	5C9
54125	71	Autoantigen calreticulin		p0020r16c20	R0077 H10	2.09	0.16	0.076	5D1
54143	72	Human hepatic squalene synthetase		p0021r09c21	R0080 A11	2.16	0.132	0.061	5E 7
54139	73	Human RAD21 homolog		p0021r05c12	R0079 B6	2.26	0.06	0.026	5E 3
54137	74	Human MHC class II HLA-DR-alpha		p0021r05c08	R0079 B4	2.16	0.097	0.045	5E 1
54044	75	Human Claudin-7		p0018r10c12	R0068 D6	5.03	0.277	0.055	4E 4

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54042	76	Ribosome protein S6 kinase 1		p0018r09c20	R0068 B10	3.56	0.116	0.033	4E 2
54043	77	CO-029 tumor associated antigen	Colon/Pancreatic	p0018r10c11	R0068 C6	2.65	0.18	0.068	4E 3
54157	79	Human lipocortin II		p0022r02c18	R0082 D9	3.84	0.265	0.069	5F9
54116	84	Tumor antigen L6		p0020r10c03	R0076 C2	2	0.105	0.052	5C4
54151	85	UDP-N-acetylglucosamine transporter		p0021r14c15	R0081 C8	2.35	0.093	0.04	5F3
54115	87	Cystine/glutamate transporter		p0020r09c16	R0076 B8	2.05	0.033	0.016	5C3
54155	89	GAPDH		p0022r01c04	R0082 B2	4.23	0.417	0.099	5F7
54169	92	Neutrophil lipocalin		p0022r15c24	R0085 F12	2.74	0.216	0.079	5G9
54167	93	Nuclear matrix protein NRP/B		p0022r13c20	R0085 B10	2.38	0.084	0.035	5G7
54163	97	Poly A binding protein		p0022r06c14	R0083 D7	3.28	0.262	0.08	5G3

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54164	98	Ribosome protein L13		p0022r08c13	R0083 G7	2.01	0.105	0.052	5G4
54132	99	Human alpha enolase		p0021r03c13	R0078 E7	2.96	0.292	0.099	5D8
54112	100	Human E-1 enzyme		p0020r08c03	R0075 G2	2.06	0.097	0.047	5B12
54165	102	Human ZW10 interactor Zwint		p0022r09c22	R0084 B11	2.46	0.055	0.022	5G5
54158	103	Bumetanide-sensitive Na-K-Cl cotransporter		p0022r03c20	R0082 F10	2.61	0.241	0.092	5F10
54108	108	NADH-ubiquinone oxidoreductase NDUF52 subunit		p0020r06c11	R0075 C6	2.07	0.105	0.051	5B8
54175	109	Phospholipase A2		p0023r04c03	R0086 G2	3.28	0.187	0.057	5H3
54177	111	Ig heavy chain variable region		p0023r05c08	R0087 B4	2.31	0.117	0.051	5H5
54170	112	Protein phosphatase 2C gamma		p0022r16c04	R0085 H2	2.03	0.136	0.067	5G10
54176	113	Cyclin protein		p0023r04c06	R0086 H3	2.12	0.165	0.078	5H4

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
54180	114	Transgelin 2 (predicted)		p0023r09c09	R0088 A5	2.21	0.166	0.075	5H8
53897	115	Human GalNAC-T3 gene		p0016r01c11	R0058 A6	2.46	0.179	0.073	3A1
54107	118	Alpha topoisomerase truncated form		p0020r05c22	R0075 B11	2.64	0.108	0.041	5B7
53902	120	AD022 protein		p0016r03c04	R0058 F2	2.3	0.123	0.053	3A6
54004	127	Cytochrome P450 IIIA4 82%		p0017r12c21	R0064 G11	2.07	0.134	0.065	4A12
53913	128	CEA		p0016r05c23	R0059 A12	5.48	0.338	0.062	3B5
54134	129	Protein phosphatase (KAP1)		p0021r04c05	R0078 G3	2.05	0.138	0.067	5D10
53938	131	Alpha enolase		p0016r12c15	R0060 G8	3.04	0.299	0.098	3D6
53939	132	Histone deacetylase HD1		p0016r12c23	R0060 G12	2.37	0.17	0.072	3D7
53914	134	Human squalene epoxidase		p0016r06c03	R0059 C2	2.12	0.07	0.033	3B6

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53915	135	Human aspartyl-tRNA-synthetase alpha-2 subunit		p0016r06c09	R0059 C5	2.02	0.121	0.06	3B7
54101	136	Gamma-actin		p0020r02c20	R0074 D10	2.91	0.21	0.072	5B1
53922	137	Human AP-mu chain family member mu1B		p0016r07c21	R0059 E11	2.07	0.161	0.078	3C2
54023	138	Human Cctg mRNA for chaperonin		p0018r02c21	R0066 C11	2.87	0.192	0.067	4C7
53921	140	Human MEGF7		p0016r07c20	R0059 F10	2.5	0.109	0.044	3C1
54002	141	Connexin 26		p0017r12c15	R0064 G8	2.13	0.133	0.063	4A10
54003	142	Human dipeptidyl peptidase IV		p0017r12c16	R0064 H8	2	0.081	0.04	4A11
53925	144	Human 2-oxoglutarate dehydrogenase		p0016r08c16	R0059 H8	2.7	0.167	0.062	3C5

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53927	145	Rho guanine nucleotide-exchange factor		p0016r09c12	R0060 B6	2.13	0.194	0.091	3C7
53937	147	Human colon mucosa-associated mRNA	Normal colon	p0016r11c23	R0060 E12	2.89	0.153	0.053	3D5
53919	151	Human embryonic lung protein		p0016r07c16	R0059 F8	2.19	0.153	0.07	3B11
53972	153	Human leukocyte surface protein CD31		p0017r04c18	R0062 H9	2.08	0.052	0.025	3G4
54144	156	Poly A binding protein		p0021r09c24	R0080 B12	2.99	0.163	0.055	5E 8
53929	158	Cystic fibrosis transmembrane conductance regulator		p0016r10c02	R0060 D1	4.15	0.181	0.044	3C9
54099	163	Human set gene		p0020r02c07	R0074 C4	2.19	0.133	0.061	5A11
53943	164	Human pleckstrin 2		p0016r14c15	R0061 C8	3	0.155	0.052	3D11
54100	166	Tis11d gene		p0020r02c09	R0074 C5	2.2	0.183	0.083	5A12

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53940	167	Cytokine (GRO-gamma)		p0016r13c17	R0061 A9	2.37	0.183	0.077	3D8
53941	168	Human p85Mcm mRNA		p0016r13c23	R0061 A12	2.25	0.09	0.04	3D9
54007	170	SOX9		p0017r13c19	R0065 A10	2.32	0.228	0.098	4B3
53950	171	VAV-like protein		p0016r15c14	R0061 F7	2.41	0.064	0.026	3E 6
53968	172	NF-E2 related factor 3		p0017r04c10	R0062 H5	2.19	0.1	0.046	3F12
54092	176	Human argininosuccinate synthetase		p0019r15c10	R0073 F5	2.73	0.199	0.073	5A4
54095	177	Human serine kinase		p0019r16c14	R0073 H7	2.57	0.126	0.049	5A7
53967	179	Human phospholipase C beta 4		p0017r04c08	R0062 H4	2.87	0.182	0.063	3F11
54032	181	VAV-3 protein		p0018r08c01	R0067 G1	2.16	0.096	0.044	4D4
54135	184	Calcium-binding protein S100P		p0021r04c13	R0078 G7	5.65	0.474	0.084	5D11
53969	185	Human leupaxin		p0017r04c14	R0062 H7	2.12	0.042	0.02	3G1



Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53970	186	VAV-3 protein		p0017r04c15	R0062 G8	2.9	0.123	0.042	3G2
53995	188	hnRNP type A/B protein		p0017r11c23	R0064 E12	2.31	0.106	0.046	4A3
54075	189	Human cell cycle control gene CDC2		p0019r04c06	R0070 H3	3.57	0.222	0.062	4G11
54096	195	Human glutamyl-tRNA synthetase		p0019r16c15	R0073 G8	2.17	0.206	0.095	5A8
54110	196	Human 26S proteasome-associated pad 1 homolog		p0020r07c22	R0075 F11	2.37	0.187	0.079	5B10
53920	197	Human squalene epoxidase		p0016r07c18	R0059 F9	3	0.205	0.068	3B12
53979	198	Human nuclear chloride ion channel protein		p0017r05c16	R0063 B8	2.2	0.116	0.053	3G11
53986	201	Human ephrin		p0017r08c09	R0063 G5	2.15	0.212	0.099	3H6
53985	205	CD9 antigen		p0017r08c06	R0063 H3	3.2	0.315	0.099	3H5
54012	207	Cyclin B		p0017r14c19	R0065 C10	2.73	0.156	0.057	4B8

Clone	SEQ ID NO.	Genbank	EST	Element (384)	Element (96)	Ratio	Median Signal 1	Median Signal 2	96 Well Location
53990	210	Colon mucosa-associated mRNA		p0017r09c22	R0064 B11	2.27	0.116	0.051	3H10

## EXAMPLE 2

### C907P IS OVEREXPRESSED IN COLON TUMORS

Using the C907P cDNA sequence, which was discovered from the subtracted cDNA library and cDNA microarray discussed above, the Genbank database was searched. C907P matches with a known gene named Epiregulin (Genbank accession number D30783). Two gene-specific primers were synthesized, and used for PCR amplification to clone this gene from colon cDNAs. The amplified PCR product was sequenced to confirm its identity. Thus, C907P-Epiregulin is a gene up-regulated in colon cancer. PCR was performed under conditions of denaturing cDNA at 94°C for 1 minute, then 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 2 minutes. Proof-reading polymerase was used for the amplification. The cDNA templates used for the PCR were synthesized from colon tumor mRNA. The amplified products were cloned into the TA cloning vector and the sequences were determined. The C907P DNA sequence is shown in SEQ ID NO:234, and the amino acid sequence is shown in SEQ ID NO:235.

## EXAMPLE 3

### FULL LENGTH PCR AMPLIFICATION AND cDNA CLONING OF THE C915P COLON TUMOR ANTIGEN

The C915P cDNA sequence (SEQ ID NO:33; also referred to by clone identifier number 54160), discovered from the subtracted cDNA library and cDNA microarray discussed in Example 1, was used to search the Genbank database. C915P was found to have some degree of similarity to a known gene named superoxidegenerating oxidase Mox1 (Genbank accession number AF127763). Two gene-specific primers were designed according to the sequence deposited in Genbank in order to amplify the full-length cDNA. PCR was performed under conditions of denaturing cDNA at 94°C for 1 minute, then 35 cycles of 94°C for 30 second, 60°C for 30 second, 72°C for 2 minutes. Proofreading polymerase was used for the amplification. The cDNA templates used for the PCR were synthesized from colon tumor mRNA. The amplified products were cloned into the TA cloning vector (Invitrogen, Carlsbad, CA) and random clones sequenced by automatic DNA

sequencing to confirm identity. The full-length cDNA and amino acid sequence of C915P is set forth in SEQ ID NO:244 and 245, respectively.

Expression levels of C915P cDNA were further analyzed by real-time PCR. Using this analysis, C915P was confirmed to be overexpressed in colon tumors as compared to a panel of normal tissues. Moderate levels of expression were observed in normal colon tissues. Real-time PCR (*see* Gibson et al., *Genome Research* 6:995-1001, 1996; Heid et al., *Genome Research* 6:986-994, 1996) is a technique that evaluates the level of PCR product accumulation during amplification. This technique permits quantitative evaluation of mRNA levels in multiple samples. Briefly, mRNA was extracted from colon tumor and normal tissue and cDNA was prepared using standard techniques. Real-time PCR was performed using a Perkin Elmer/Applied Biosystems (Foster City, CA) 7700 Prism instrument. Matching primers and a fluorescent probe were designed for C915P using the primer express program provided by Perkin Elmer/Applied Biosystems (Foster City, CA). Optimal concentrations of primers and probe were initially determined and control (*e.g.*,  $\beta$ -actin) primers and probe were obtained commercially. To quantitate the amount of specific RNA in a sample, a standard curve was generated using a plasmid containing the C915P cDNA. Standard curves were generated using the Ct values determined in the real-time PCR, which are related to the initial cDNA concentration used in the assay. Standard dilutions ranging from  $10^{-10}$  copies of the C915P were generally sufficient. In addition, a standard curve was generated for the control sequence. This permitted standardization of initial RNA content of the tissue samples to the amount of control for comparison purposes.

25

#### EXAMPLE 4

##### PRODUCTION OF RA12-C915P-F3 RECOMBINANT PROTEIN IN *E. COLI*

C915P (also referred to as clone identifier 54160, and set forth in SEQ ID NOs:33 and 244 (cDNA), and 245 (amino acid)) has 6 transmembrane domains (TMs) with 3 extracellular loops (ED1, ED2, and ED3). The deletion recombinant protein, Ra12-C915P-f3 (set forth in SEQ ID NOs:236 (cDNA) and 237 (amino acid)),

30

is an N-terminal Ra12 fusion of recombinant, modified C915P in pCRX1 vector (EcoR I, Xho I).

Cloning Strategy for Ra12-C915P-f3:

Three sets of primers were designed that were used sequentially to delete  
5 two internal transmembrane domains and amplify a recombined internal region of C915P that was cut with EcoRI and XhoI and ligated in frame with Ra12 in the pCRX1 vector.

PCR#1 used primers AW157 and AW156 (SEQ ID NO:241 and 240, respectively) to amplify the entire construct, deleting TM4 - ID3 - TM5. The PCR  
10 product (C915P(minusTM4-ID3-TM5) PCR Blunt II TOPO backbone) was purified from agarose gel, ligated by T4 DNA Ligase and transformed into NovaBlue *E. coli* cells with the following standard protocol: the competent *E. coli* cells were thawed on ice, DNA (or ligation mixture) was added, the reaction mixed and incubated on ice for 5 minutes. The *E. coli* cells were heat-shocked at 42°C for 30 seconds, and left on ice for  
15 2 minutes. Enriched growth media was added to the *E. coli* and they were grown at 37°C for 1 hour. The culture was plated on LB (plus appropriate antibiotics) and grown overnight at 37°C. The next day, several colonies were randomly selected for miniprep (Promega, Madison, WI) and were confirmed by DNA sequencing for correctly deleted region. This step was then repeated on a second region of C915P as described below.

20 PCR#2 used primers AW155 and AW154 (SEQ ID NOs:239 and 238, respectively) to delete TM2, using a confirmed clone from PCR#1 as template. The PCR product (C915P(minusTM2 / TM4-ID3-TM5) PCR Blunt II TOPO backbone) was purified, ligated and transformed using standard protocols into NovaBlue cells, yielding clones that were confirmed by sequencing for the correct deletion.

25 PCR#3 used primers AW158 and AW159 (SEQ ID NOs:242 and 243, respectively) to amplify the deleted, recombined three-part fusion protein of C915P, ED1 - ID2-TM3-ED2 - ED3, using the confirmed PCR#2 clone as template. PCR product from PCR#3 was purified and digested using EcoR I and Xho I for ligation into the pCRX1 vector (EcoR I, Xho I). The ligation mixture was transformed into  
30 NovaBlue cells by standard protocols, and several clones were selected for miniprep

and sequencing. UI#70526 was confirmed by DNA sequencing to be the correct pCRX1 Ra12-C915P-f3 construct.

**Cloning Primers:**

5 C915P-AW154 (SEQ ID NO:238): antisense cloning primer to delete TM2, 5'P—Primer Id9682: 5' P- TTTTCTTGTGTAGTAGTATTTGTGCG.

C915P-AW155 (SEQ ID NO:239): sense cloning primer to delete TM2, 5'P—Id 9683: 5' P-TGTCGCAATCTGCTGTCCTTCC.

10 C915P-AW156 (SEQ ID NO:240): antisense cloning primer to delete TM4-TM5 region, 5'-P, --Primer Id 9684: 5' P- GCTGGTGAATGTCACATACTCC.

C915P-AW157 (SEQ ID NO:241): sense cloning primer to delete TM4-TM5 region, 5'-P – Id 9685: 5' P- CGGGGTCAAACAGAGGAGAG.

15 Ra12-C915P-F3-AW158 (SEQ ID NO:242): sense cloning primer for the fusion protein with EcoR I site Primer Id 9686: 5' gtcgaattcGATGCCTTCCTGAAATATGAGAAG.

Ra12-C915P-F3-AW159 (SEQ ID NO:243): antisense cloning primer for the fusion protein with stop and Xho I site – Primer Id 9687: 5' cacctcgagttaAGACTCAGGGGGATGCCCTTC.

Protein Information for Ra12-C915P-f3:

20 Molecular Weight 32429.45 Daltons  
297 Amino Acids  
28 Strongly Basic(+) Amino Acids (K,R)  
27 Strongly Acidic(-) Amino Acids (D,E)  
93 Hydrophobic Amino Acids (A,I,L,F,W,V)  
25 86 Polar Amino Acids (N,C,Q,S,T,Y)  
7.776 Isoelectric Point  
3.711 Charge at PH 7.0

Protein Expression:

30 Mini expression screens were performed to determine the optimal induction conditions for Ra12-C915P-f3. The best *E. coli* strain/culture conditions

were screened by transforming the expression construct into different hosts, then varying temperature, culture media and/or IPTG concentration after the inducer IPTG was added to the mid-log phase culture. The recombinant protein expression was then analyzed by SDS-PAGE and/or Western blot. *E. coli* expression hosts BLR (DE3) and  
5 HMS (DE3) (Novagen, Madison, WI) were tested in various culture conditions, with little full-length Ra12-C915P-f3 expression detected and Western blots showing some bands at unexpected molecular weights. Tuner (DE3) cells (Novagen, Madison, WI) were then tested with helper plasmids at various IPTG concentrations. Coomassie stained SDS-PAGE showed no induced band but Western blot confirmed a strong  
10 Ra12-C915P-f3 signal at 32kD probing with an anti-6xhis tag antibody. The most optimal expression for pCRX1 Ra12-C915P-f3 was found to be in the host strain Tuner (DE3) with a helper plasmid grown in Soy Terrific Broth media at 37°C induced with 1.0 mM IPTG at 37°C for 3hr.

15

#### EXAMPLE 5

##### PURIFICATION OF RA12-C915P-F3 RECOMBINANT FUSION PROTEIN FROM *E. COLI*

The clone C915P was found to be over-expressed in a majority of colon cancer tissues. For expression in *E. coli*, the construct Ra12-C915P-f3 (SEQ ID NO:236) was made as described in Example 4. This construct encodes a fusion protein  
20 consisting of an N-terminal 6x histidine tag followed by Ra12 and modified C915P (excluding 5 of 6 transmembrane domains) (SEQ ID NO:237). The 32.4kD protein was expressed in multiple large baffled shaker flasks containing 1L of Soy Terrific Broth media. The cultures were spun and cell pellets washed, respun and frozen for purification. After cell lysis, the recombinant protein was found in the insoluble  
25 inclusion body fraction. The inclusion body was thoroughly washed with buffered detergents multiple times, then the protein pellet was denatured, reduced and solubilized in buffered 8M Urea and Ra12-C915P-f3 protein was bound to a Ni-NTA affinity chromatography matrix. The matrix was washed to rinse away contaminating  
30 *E. coli* proteins and Ra12-C915P-f3 was subsequently eluted using high Imidazole concentration. The fractions containing Ra12-C915P-f3 were pooled and slowly dialyzed to allow for renaturation of the protein. The purified Ra12-C915P-f3 was then

filtered and quantified. SDS-PAGE analysis showed the elution pattern off the nickel column with the major band running at the expected weight of about 32kD. This was further confirmed by western blot using an anti-6x His tag antibody. The western blot also revealed evidence of dimers and tetramers of the recombinant. N-terminal  
5 sequencing confirmed purity of about 90%. Purified yield was about 2.5 mg/L induction.

Following is a detailed protocol of the production of purified Ra12-C915P-f3.

For the frozen bacterial cell pellet:

- 10 1. Thaw bacterial cell pellet from 1L induction on ice
2. Add 25ml sonication buffer (20mM Tris, 500mM NaCl) per liter of induction culture
3. Add 1 Complete protease inhibitor tablet and 2mM PMSF (Phenylmethylsulfonyl fluoride) to sonication buffer/pellet mix
- 15 4. Completely resuspend pellet with pipet
5. Add 0.5mg/ml lysozyme (made fresh from lyophilized lysozyme stored at -20°C)
6. Decant into a glass beaker + stir bar, gently stir at 4°C, 30 min
7. French Press 2 x 1100psi, keep on ice
- 20 8. Once lysis solution\*\* has low viscosity, spin at 11000RPM, 30min, 4°C
9. Save supernatant\*\* and pellet

For the pellet from step 9 above:

- 25 1. Wash pellet with 25ml 0.5% CHAPS (3-([3-Cholamidopropyl]dimethylammonio)-1-propanesulfonate) wash (20mM Tris (8.0), 500mM NaCl) \*\* by sonicating 2x15sec @15Watt
2. Spin at 11000RPM for 25min. Repeat 5x\*\*
- 30 3. Repeat above steps 3 times with 0.5% DOC (Deoxycholic Acid) wash (20mM Tris (8.0), 500mM NaCl)



4. Resuspend pellet in pellet binding buffer (20mM Tris (8.0), 500mM NaCl, 8M Urea, 20mM Imidazole, 10mM  $\beta$ -Mercaptoethanol) with sonication
5. Equilibrate Ni ++ NTA (Nitrilotriacetic acid) resin (Qiagen, Valencia, CA) with pellet binding buffer, spin down and decant wash (use 4ml resin)
6. Add resin to resuspended pellet, stir at room temperature for 45min
7. Prepare column and buffers, rinse column with pellet binding buffer
8. Pour pellet/Ni resin into column, collect flow through (FT)\*\*
9. Wash column with 30ml pellet binding buffer \*\*
10. Wash column with 30ml pellet binding buffer with 0.5% DOC (Deoxycholic Acid)\*\*
11. Wash column with 30ml pellet binding buffer
12. Elute with 5 x 5ml fractions of pellet binding buffer #1 (binding buffer +300mM Imidazole)\*\*
13. Elute with 2 x 5ml fractions of pellet elution buffer #2 (binding buffer +300mM Imidazole, pH 4.5)\*\*
14. Run SDS-PAGE to screen purification steps (western and coomassie stain)

\*\*Save an aliquot at 4°C for each purification step to check on SDS-PAGE.

#### EXAMPLE 6

##### REAL-TIME PCR ANALYSIS OF COLON TUMOR CANDIDATE GENES

The first-strand cDNA to be used in the quantitative real-time PCR was synthesized from 20 $\mu$ g of total RNA that had been treated with DNase I (Amplification Grade, Gibco BRL Life Technology, Gaithersburg, MD), using Superscript Reverse Transcriptase (RT) (Gibco BRL Life Technology, Gaithersburg, MD). Real-time PCR

was performed with a GeneAmp™ 5700 sequence detection system (PE Biosystems, Foster City, CA). The 5700 system uses SYBR™ green, a fluorescent dye that only intercalates into double stranded DNA, and a set of gene-specific forward and reverse primers. The increase in fluorescence is monitored during the whole amplification process. The optimal concentration of primers was determined using a checkerboard approach and a pool of cDNAs from breast tumors was used in this process. The PCR reaction was performed in 25µl volumes that include 2.5µl of SYBR green buffer, 2µl of cDNA template and 2.5µl each of the forward and reverse primers for the gene of interest. The cDNAs used for RT reactions were diluted 1:10 for each gene of interest and 1:100 for the β-actin control. In order to quantitate the amount of specific cDNA (and hence initial mRNA) in the sample, a standard curve is generated for each run using the plasmid DNA containing the gene of interest. Standard curves were generated using the Ct values determined in the real-time PCR which were related to the initial cDNA concentration used in the assay. Standard dilution ranging from 20-2x10<sup>6</sup> copies of the gene of interest was used for this purpose. In addition, a standard curve was generated for β-actin ranging from 200fg-2000fg. This enabled standardization of the initial RNA content of a tissue sample to the amount of β-actin for comparison purposes. The mean copy number for each group of tissues tested was normalized to a constant amount of β-actin, allowing the evaluation of the over-expression levels seen with each of the genes.

Colon tumor candidate genes, C906P (SEQ ID NO:5), C907P (SEQ ID NO:234 (cDNA) and 235 (amino acid)), C911P (SEQ ID NO:21), C915P (SEQ ID NO:244 (cDNA) and 245 (amino acid)), C943P (SEQ ID NO:140), and C961P (SEQ ID NO:200), were analyzed by real-time PCR, as described above, using the short and extended colon panel. These genes were found to have increased mRNA expression in 30-50% of colon tumors. For C906P, slightly elevated expression was also observed in normal trachea, heart, and normal colon. For C907P, elevated expression was also observed in activated PBMC and slightly elevated expression in heart and normal colon. For C911P, slightly elevated expression was observed in pancreas. For C915P, no expression was observed in normal tissues except normal colon. For C943P, no expression was observed in normal tissues except normal colon. For C961P, some

expression was observed in trachea and normal colon. Collectively, the data indicate that these colon tumor candidate genes could be potential targets for immunotherapy and cancer diagnosis.

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#### EXAMPLE 7

##### PEPTIDE PRIMING OF T-HELPER LINES

Generation of CD4<sup>+</sup> T helper lines and identification of peptide epitopes derived from tumor-specific antigens that are capable of being recognized by CD4<sup>+</sup> T cells in the context of HLA class II molecules, is carried out as follows:

10 Fifteen-mer peptides overlapping by 10 amino acids, derived from a tumor-specific antigen, are generated using standard procedures. Dendritic cells (DC) are derived from PBMC of a normal donor using GM-CSF and IL-4 by standard protocols. CD4<sup>+</sup> T cells are generated from the same donor as the DC using MACS beads (Miltenyi Biotec, Auburn, CA) and negative selection. DC are pulsed overnight  
15 with pools of the 15-mer peptides, with each peptide at a final concentration of 0.25 µg/ml. Pulsed DC are washed and plated at 1 x 10<sup>4</sup> cells/well of 96-well V-bottom plates and purified CD4<sup>+</sup> T cells are added at 1 x 10<sup>5</sup>/well. Cultures are supplemented with 60 ng/ml IL-6 and 10 ng/ml IL-12 and incubated at 37°C. Cultures are restimulated as above on a weekly basis using DC generated and pulsed as above as  
20 antigen presenting cells, supplemented with 5 ng/ml IL-7 and 10 U/ml IL-2. Following 4 *in vitro* stimulation cycles, resulting CD4<sup>+</sup> T cell lines (each line corresponding to one well) are tested for specific proliferation and cytokine production in response to the stimulating pools of peptide with an irrelevant pool of peptides used as a control.

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#### EXAMPLE 8

##### GENERATION OF TUMOR-SPECIFIC CTL LINES USING IN VITRO WHOLE-GENE PRIMING

Using *in vitro* whole-gene priming with tumor antigen-vaccinia infected DC (see, for example, Yee et al, *The Journal of Immunology*, 157(9):4079-86, 1996),  
30 human CTL lines are derived that specifically recognize autologous fibroblasts transduced with a specific tumor antigen, as determined by interferon-γ ELISPOT

analysis. Specifically, dendritic cells (DC) are differentiated from monocyte cultures derived from PBMC of normal human donors by growing for five days in RPMI medium containing 10% human serum, 50 ng/ml human GM-CSF and 30 ng/ml human IL-4. Following culture, DC are infected overnight with tumor antigen-recombinant vaccinia virus at a multiplicity of infection (M.O.I) of five, and matured overnight by the addition of 3 µg/ml CD40 ligand. Virus is then inactivated by UV irradiation. CD8+ T cells are isolated using a magnetic bead system, and priming cultures are initiated using standard culture techniques. Cultures are restimulated every 7-10 days using autologous primary fibroblasts retrovirally transduced with previously identified tumor antigens. Following four stimulation cycles, CD8+ T cell lines are identified that specifically produce interferon-γ when stimulated with tumor antigen-transduced autologous fibroblasts. Using a panel of HLA-mismatched B-LCL lines transduced with a vector expressing a tumor antigen, and measuring interferon-γ production by the CTL lines in an ELISPOT assay, the HLA restriction of the CTL lines is determined.

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#### EXAMPLE 9

##### GENERATION AND CHARACTERIZATION OF ANTI-TUMOR ANTIGEN MONOCLONAL ANTIBODIES

Mouse monoclonal antibodies are raised against *E. coli* derived tumor antigen proteins as follows: Mice are immunized with Complete Freund's Adjuvant (CFA) containing 50 µg recombinant tumor protein, followed by a subsequent intraperitoneal boost with Incomplete Freund's Adjuvant (IFA) containing 10µg recombinant protein. Three days prior to removal of the spleens, the mice are immunized intravenously with approximately 50µg of soluble recombinant protein. The spleen of a mouse with a positive titer to the tumor antigen is removed, and a single-cell suspension made and used for fusion to SP2/O myeloma cells to generate B cell hybridomas. The supernatants from the hybrid clones are tested by ELISA for specificity to recombinant tumor protein, and epitope mapped using peptides that spanned the entire tumor protein sequence. The mAbs are also tested by flow

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cytometry for their ability to detect tumor protein on the surface of cells stably transfected with the cDNA encoding the tumor protein.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

## CLAIMS

## What is Claimed:

1. An isolated polynucleotide comprising a sequence selected from the group consisting of:
  - (a) sequences provided in SEQ ID NO:1-234, 236, and 244;
  - (b) complements of the sequences provided in SEQ ID NO:1-234, 236, and 244;
  - (c) sequences consisting of at least 20 contiguous residues of a sequence provided in SEQ ID NO:1-234, 236, and 244;
  - (d) sequences that hybridize to a sequence provided in SEQ ID NO:1-234, 236, and 244, under moderately stringent conditions;
  - (e) sequences having at least 75% identity to a sequence of SEQ ID NO:1-234, 236, and 244;
  - (f) sequences having at least 90% identity to a sequence of SEQ ID NO:1-234, 236, and 244; and
  - (g) degenerate variants of a sequence provided in SEQ ID NO:1-234, 236, and 244.
2. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
  - (a) sequences encoded by a polynucleotide of claim 1;
  - (b) amino acid sequences set forth in SEQ ID NO:235, 237, and 245;
  - (c) sequences having at least 70% identity to a sequence encoded by a polynucleotide of claim 1; and
  - (d) sequences having at least 90% identity to a sequence encoded by a polynucleotide of claim 1.
3. An expression vector comprising a polynucleotide of claim 1 operably linked to an expression control sequence.

4. A host cell transformed or transfected with an expression vector according to claim 3.

5. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to a polypeptide of claim 2.

6. A method for detecting the presence of a cancer in a patient, comprising the steps of:

- (a) obtaining a biological sample from the patient;
- (b) contacting the biological sample with a binding agent that binds to a polypeptide of claim 2;
- (c) detecting in the sample an amount of polypeptide that binds to the binding agent; and
- (d) comparing the amount of polypeptide to a predetermined cut-off value and therefrom determining the presence of a cancer in the patient.

7. A fusion protein comprising at least one polypeptide according to claim 2.

8. An oligonucleotide that hybridizes to a sequence recited in SEQ ID NO:1-234, 236, and 244 under moderately stringent conditions.

9. A method for stimulating and/or expanding T cells specific for a tumor protein, comprising contacting T cells with at least one component selected from the group consisting of:

- (a) polypeptides according to claim 2;
- (b) polynucleotides according to claim 1; and
- (c) antigen-presenting cells that express a polypeptide according to claim 1,

under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells.

10. An isolated T cell population, comprising T cells prepared according to the method of claim 9.

11. A composition comprising a first component selected from the group consisting of physiologically acceptable carriers and immunostimulants, and a second component selected from the group consisting of:

- (a) polypeptides according to claim 2;
- (b) polynucleotides according to claim 1;
- (c) antibodies according to claim 5;
- (d) fusion proteins according to claim 7;
- (e) T cell populations according to claim 10; and
- (f) antigen presenting cells that express a polypeptide according to claim 2.

12. A method for stimulating an immune response in a patient, comprising administering to the patient a composition of claim 11.

13. A method for the treatment of a cancer in a patient, comprising administering to the patient a composition of claim 11.

14. A method for determining the presence of a cancer in a patient, comprising the steps of:

- (a) obtaining a biological sample from the patient;
- (b) contacting the biological sample with an oligonucleotide according to claim 8;
- (c) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; and
- (d) compare the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence of the cancer in the patient.



15. A diagnostic kit comprising at least one oligonucleotide according to claim 8.

16. A diagnostic kit comprising at least one antibody according to claim 5 and a detection reagent, wherein the detection reagent comprises a reporter group.

17. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

(a) incubating CD4+ and/or CD8+ T cells isolated from a patient with at least one component selected from the group consisting of: (i) polypeptides according to claim 2; (ii) polynucleotides according to claim 1; and (iii) antigen presenting cells that express a polypeptide of claim 2, such that T cell proliferate;

(b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient.

## SEQUENCE LISTING

<110> Corixa Corporation  
 Jiang, Yuqiu  
 Hepler, William T.  
 Clapper, Jonathan  
 Wang, Aijun  
 Secrist, Heather

<120> COMPOSITIONS AND METHODS FOR THE THERAPY  
 AND DIAGNOSIS OF COLON CANCER

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 tcaacaatat tccacagcag cccagatgca agtggaacaa caccctcatc tgccactcc 420  
 acaacctcag gtcgtggaga atctacaacc tcacgcatca gtccaggctc aactgaaata 480  
 acaacgttac ctggcagt 498

<210> 13  
 <211> 523  
 <212> DNA  
 <213> Homo sapien

<400> 13  
 accacagcat catcccttgg tccagaatat actaccttcc acagccgccc aggtccact 60

```

gaaacaacac tcttacctga caacaccaca gcctcaggac tccttgaagc atctacgccc 120
gtccacagca gcaccagatc gccacacaca acactgtccc ctgccggctc tacaacccgt 180
cagggagaat ctaccacatt ccatagctgg ccaagctcaa aggacactag gcccgcacct 240
cctactacca catcagcctt tgttaaacta tctacaactt atcacagcag cccgagctca 300
actccaacaa ccactttttc tgccagctcc acaaccttgg gccatagtga ggaatcgaca 360
ccagtcacaa gcagcccagt tgcaactgca acaacacccc cacctgcccc ctccgcgacc 420
tcaggccatg ttgaagaatc tacagcctac cacaggagcc cgggctcaac tcaaacaatg 480
cacttccctg aaagctccac aacttcaggc catagtgaag aat 523

```

```

<210> 14
<211> 461
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(461)
<223> n = A,T,C or G

```

```

<400> 14
caggtacaag tcattactcc cccttctccc atatgaacaa gaatttttta acggtcagaa 60
tatattgggc atcaaattaa aaactttttt ttcaaaagtc tacagaatgg atattggagc 120
aaaaattaca aagtgggtca gatacagggt tttaaaaaact gcattactga atttaacaaa 180
agtcagacac tagaatcata tatttgctgc ataaaagttg atttgatacc tgggtggtgat 240
tgaatttagt ctcaaagact cataaataaa aatctgactt aagacgtagt cataccagta 300
taccaattct cccatcactt tgactttcgg cagagagatt agagcaaaaa atattcagga 360
gaacagtgga gttacattgn attatgtatg tttaataata tatcaatttt aagggttaagg 420
ttaaggaaat cttaatttta agntaaacc ttgagtacct c 461

```

```

<210> 15
<211> 508
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(508)
<223> n = A,T,C or G

```

```

<400> 15
cgcggcgagg taccagtgtg tgttcgtatt tgggcacagg ctttnggggg cactgcgtt 60
gcagntgaca tgtgccagg ttacagttca ttgcgactt cgttcctttg gtgcattgt 120
tcacacaggc cagcttcccg tccaagacat ccacatagta gaactgggta tatccttcgg 180
cagccttctg ggtgcattgc tcctggaagt caaagcccg agtcaccgat gaatccacga 240
aagtgtcctc ttcactatag cacagtatgg cctttctgca ggaatcagga tcaagaagag 300
ttgttctagt ttcattcata atcttggcct ttacaatctc tgccaggttt tcaaacagtt 360
cctcactatc taaagtgtag tctgcctcca ggatgacatc gttcttgacc acgatgtctac 420
cgttgagcaa tctccgaatg ttcaccctc tatactgagg aagattgtcg cccttcaaaa 480
cgacatccat ccgattcttg aagagggt
508

```

```

<210> 16
<211> 578
<212> DNA
<213> Homo sapien

```

```

<400> 16
acatatataat gaatctggtg ttggggaaac cttcatctga aaccacaga tgtctctggg 60
gcagatcccc actgtectac cagttgccct agcccagact ctgagctgct caccggagtc 120

```

```

attgggaagg aaaagtggag aaatggcaag tctagagtct cagaaactcc cctggggggtt 180
tcacctgggc cctggaggaa ttcagctcag ctctctccta ggtccaagcc cccacacact 240
tttcccaaac cacagagaac aagagtttgt tctgttctgg gggacagaga aggcgcttcc 300
caacttcata ctggcaggag ggtgaggagg ttcactgagc tcccagatc tccactgcg 360
gggagacaga agcctggact ctgccccacg ctgtggccct ggagggtccc ggtttgtcag 420
ttcttggtgc tctgtgttcc cagaggcagg cggagggtga agaaaggaac ctgggatgag 480
gggtgctggg tataagcaga gagggatggg ttctgtctcc aagggaccct ttgcctttct 540
tctgcccttt cctaggccca ggcctggggt tgtacctt 578

```

```

<210> 17
<211> 623
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(623)
<223> n = A,T,C or G

```

```

<400> 17
acacagaagt ttgaatcaca aaacataatt accacaataa aacacagtgt tcaagtatct 60
tggcagagca atctgccgca caaactgcaa attaaattaa ctacacagac taaaaactat 120
acagcctacc atcaacagtt gtgcattata aaaaggtagt ttctttcctt ttgttttaag 180
tcaggaacag gtagattttt aaaaatatat atacaagcta acacacacag ctatcagcac 240
taatgcccc ccctcaactt ttctttttt ttatagaaaa tggaaagctt acaatacctc 300
ctccatcaaa ggggcaggcc tacgagccag cctgaacagg gtttgccttg gaaaagatgt 360
ggcctgaggt ttagagccgc tttgtgcggg gatggtggag gctagggtgg ggtgagaaa 420
agggagaagg cggaaggggg acggacagtt ctctcttagt tacccttttt 480
tctaaataag cccaaatggc atcaactcgt ttttgctcgg tctttgttga tttcttcat 540
tttcacctcg cggttctgga accagatctt gacctgctct cggtgagggt gagcagtcga 600
gccctcgtta cctgccggcg gnc 623

```

```

<210> 18
<211> 477
<212> DNA
<213> Homo sapien

```

```

<400> 18
acacaaaagg gcatagtcct acaaagtgtt ttatataatt gttttatgtg tgcaaattga 60
aatattaaag atggatcagg gatctcagtt taaggaatcc tgccttctgt atgatgatgt 120
cttaattttt gagattttca tatattgggt tatagctata tatcaggaca ggtaaataca 180
ttataaaatt ataaccttta taataatttt tagtataatc acttggtgtga ctataataaa 240
ttggcttttag ttttctttac tcttcacagt tttaataggt aactatttta caagaataac 300
attgctaggt agaaaaattt ctgttcagtt aggagttctt attttgctgc tgaaatgagt 360
catgcacaat tttaaatctc tgtagtttct tcataagcta ttttactatc ttactatttt 420
ataagccttg tgttgcagtc aagtttttac cacattctat agaccttgct gtacctg 477

```

```

<210> 19
<211> 374
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(374)
<223> n = A,T,C or G

```

```

<400> 19

```

agaaacttta	gcattggccc	agtagtggct	tctagctcta	aatgtttgcc	ccgccatccc	60
tttccacagt	atccttcttc	cctcctcccc	tgtctctggc	tgtctcgagc	agtctagaag	120
agtgcatttc	cagcctatga	aacagctggg	tctttggcca	taagaagtaa	agatttgaag	180
acagaaggaa	gaaactcagg	agtaagcttc	tagacccctt	cagcttctac	acccttctgc	240
cctctctcca	ttgcctgcac	cccaccccag	ccactcaact	cctgcttggt	tttcctttgg	300
ccataggaag	gtttaccagt	agaatccttg	ctaggttgat	gtgggccata	cattccttta	360
ataaaccatt	gngt					374

&lt;210&gt; 20

&lt;211&gt; 207

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 20

acaagtgtgg	cctcatcaag	ccctgcccag	ccaactactt	tgcgtttaaa	atctgcagtg	60
gggccgccaa	cgctcgtggc	cctactatgt	gctttgaaga	ccgcatgac	atgagtcctg	120
tgaaaaacaa	tgtgggcaga	ggcctaaaca	tcgccctggt	gaatggaacc	acgggagctg	180
tgctgggaca	gaaggcattt	gacatgt				207

&lt;210&gt; 21

&lt;211&gt; 557

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 21

acaaagaatc	cctagacgcc	atactgagtt	ttaagttcct	taattcctaa	ttaaggctt	60
ctagtgaagc	ctcctcacag	taggcttcac	taggccaca	gtgccctag	acctctgaca	120
atcccaccct	agacagactt	tattgcaaaa	tgcgccctga	gaggcagatg	attcccaaga	180
gaactcacca	aatcaagaca	aatgtcctag	atctctagtg	tggtagaact	atgcacctaa	240
acattgctgc	aaaatgaaca	cactttttaga	cacccttgca	gatattctaag	taagtggaga	300
agactatttt	ttcaacaaac	atthtctctt	tcaccctaac	tcctaaacag	cttactgggg	360
cttctgcaag	acagaaagat	cataattcag	aaggtaacca	tcgttataga	cataaagttt	420
ctggtcaaaa	gggttatagt	taatgctctg	cactttttcc	tgcatcttat	gcattacaat	480
gtctagtttg	ccctctttcc	ctgtgtttgt	gtcataatag	taaaaaatct	cttctgttct	540
ggggtcatag	cacctcg					557

&lt;210&gt; 22

&lt;211&gt; 541

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 22

acctagggtgc	tagtctcccc	actaactgag	ggaaaaaggt	tcccaggtgg	ggtcctctgc	60
ccactttggc	accacattca	cattccaaat	gggataatgc	ctgagggggc	aagagtggtc	120
aggctgccct	ggggtgaatg	tcacctgat	gaggcccatc	agctcttgcc	cactcagtg	180
ggccagactt	gtgctcta	ccactctcct	gtgggtccct	ggcctgtatg	gcttatactg	240
gggagctggg	cctctgggct	gtccaaaccc	aagggtcaca	ctttgctttt	cctttgttgt	300
ccccattttc	catccttgct	ctaagacaaa	acttttccca	gagaagaact	ctttgttgct	360
cccgctcagc	tgtaattctg	ccttttctac	cttcattcca	tccttcctct	gccagataa	420
agtccagcag	aaattcctcc	tttctacctc	tctgggactc	tgagacagga	aatcttcaag	480
gaggagtttt	tccctcccca	ctattcttat	tctcaacccc	cagaggaacc	aaggctgctg	540
t						541

&lt;210&gt; 23

&lt;211&gt; 486

&lt;212&gt; DNA

&lt;213&gt; Homo sapien



```

<400> 23
acaaaaattgt  tggaatttag  ctaatagaaa  aacatagtaa  atatttaca  aaacgttgat  60
aacattactc  aagtcacaca  catataacaa  tgtagacagg  tcttaacaaa  gtttacaat  120
tgaaattatg  gagatttccc  aaaatgaatc  taatagctca  ttgctgagca  tggttatcaa  180
tataacattt  aagatcttgg  atcaaatggt  gtccccgagt  cttctacaat  ccagtcctct  240
tagaaattgg  tttctctctt  tgggagattc  agactcagag  gcagccagag  gggacaggtc  300
aagagctgaa  ataatcacat  aactactcta  attttcttca  ttctattgac  tgtgtcaagt  360
tatagacaca  gccaaagtgt  ttttcttcgg  cctctgatga  tttgagaaga  tgaagaacat  420
gagcaatttc  tcattgctta  aagaaaaact  tggcacataa  gaggctgagt  gtagtagagt  480
atctgt  486

```

```

<210> 24
<211> 450
<212> DNA
<213> Homo sapien

```

```

<400> 24
actgatacat  gctataacag  agatgaactt  cgaaaacatg  ctaagtgaaa  gaagccaaat  60
ccaaaaacaa  taaaaacaca  tattgtatcc  tcaccctttt  cgcattttag  tgagcaatca  120
ttgcatatga  atgttttatg  gaaaaatcaa  tgtgtgctaa  atcattgtat  tccagtaaat  180
agattggact  taaaacttga  tacagaagtt  gcaataaagt  gggattgagt  ttgattatta  240
tatagaaaat  aattacatga  ttcatttaag  aataataata  tccaccattt  attgagcact  300
tactatgagc  ctgtgtgcca  aacatttcat  gcatttctca  ttttaattctc  acaataatcc  360
tgtgaggtag  aagctattag  gttgaatcat  atgaacttgc  caatatatga  taatttctaa  420
gagttgggaa  tttttgagga  tgtgaatggt  450

```

```

<210> 25
<211> 638
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(638)
<223> n = A,T,C or G

```

```

<400> 25
gcagggtacac  gtagcgcttc  cccgacgtct  tgtggatgat  gttcttgncc  taatagtagc  60
gtaagccccg  gctcagcttc  tcgtagttca  tcttgggctt  attttctctc  ttccccacc  120
ggcgggccac  ctcatcgagg  tcggcgagct  taaactccca  tccgtctcca  gtccagctga  180
tgaatgactg  gcaggatttg  tctgatagca  gctccaggag  aaactgccac  agctgaatag  240
gtccacttcc  tgtgaagccg  gccagcacag  ctgcagggtat  aactggtttg  ccttgctcca  300
ccgggtcact  cctctcttgg  atgtaatcct  tgaaagacat  gggttgctta  ttgaggcaga  360
gagactggct  gcagtcattc  tcgaagctct  cgaaggaagg  aaccggttgc  acatccagca  420
aggacgactg  gctgttccag  gactggagga  gggagtctga  gctctcgagg  ctgtccgcac  480
cgttctcagg  ggagtcgtgg  tctttgggag  tcccagaatt  gttggtgagc  aaattcaagt  540
tgctgcctgg  gaagtcctga  ctgacagagc  agtaggtgac  gctgacggag  ctgagccgag  600
acttggggaa  catctgaaac  tinctgtcaa  agctgagt  638

```

```

<210> 26
<211> 469
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(469)
<223> n = A,T,C or G

```

<400> 26  
 naggtaccaa atggagaaaa ctctttccgg agacgttcat catcaatacc atcatcaaga 60  
 tttttcacat aaagattaac accctgggat ctgggtgatcc tatcttgttt catctgttca 120  
 aatttgcgct taagttccgt ctgccgttcc acctttttct gagctcgacc aacataaatt 180  
 tgttttccat tgagctcctt tccgttcato tcatccacag ctttctgtgc atcttcatgc 240  
 ctttcaaagc ttacaaatcc aaatcctttg gattttccac tttcatcagt cattactttc 300  
 acacttaagg caggcccaaa cttgccaaa agatccttaa ggcgctcato atccatgtct 360  
 tctccaaaat tcttgatgta aacattggtg aattcttttg cctagctcca agttcagctt 420  
 ctcgtcttta cgagacttaa atcggccaac aaatactttg cgatcattt 469

<210> 27  
 <211> 364  
 <212> DNA  
 <213> Homo sapien

<400> 27  
 actctgctat ggtgctggct tcctttaaac tcaggataga tgccagggtg gctccgtttc 60  
 cgtaagactg aactcagagc tcggcatcag accagttcct cagcttcctg aagtaacct 120  
 agcaattgga cttgtggtaa aaccatccag gagcacagct gggctcctatg atgatcac 180  
 ccaggactcc tgttttggcc aggcagctca gcaataggag cagccgcatg cttctggaag 240  
 ccatcttcc cctaccctga ggatgtagct agtgcaagga tctcagagac cttactagcg 300  
 cttctttgaa actcctgggt tctccttgat ctgcaaactt gtttggaac caagactcta 360  
 aggg 364

<210> 28  
 <211> 714  
 <212> DNA  
 <213> Homo sapien

<220>  
 <221> misc\_feature  
 <222> (1)...(714)  
 <223> n = A,T,C or G

<400> 28  
 ccttcgagaa gatccctagt gagactttga accgtatcct gggcgaccca gaagccctga 60  
 gagacctgct gaacaaccac atcttgaagt cagctatgtg tgctgaagcc atcgttgcgg 120  
 ggctgtctgt agagaccctg gagggcacga cactggaggt gggctgcagc ggggacatgc 180  
 tcactatcaa cgggaaggcg atcatctcca ataaagacat cctagccacc aacggggtga 240  
 tccactacat tgatgagcta ctcatccag actcagccaa gacactattt gaattggctg 300  
 cagagtctga tgtgtccaca gccattgacc ttttcagaca agccggcctc ggcaatcatc 360  
 tctctggaag tgagcgggtg accctcctgg ctcccctgaa ttctgtattc aaagatggaa 420  
 cccctccaat tgatgcccat acaaggaatt tgcttcggaa ccacataatt aaagaccagc 480  
 tggcctctaa gtatctgtac catggacaga ccttggaac tctgggcggc aaaaaactga 540  
 gagtttttgt ttatcgtaat agcctctgca ttgagaacag ctgcatcgcg gcccacgaca 600  
 agagggggag gtacgggacc ctgttcacga tggaccgggt gctgacccc ccaatggggg 660  
 actgtcattg gatgtcctga agggagacaa tcgcttttnc tgctggtagc tggc 714

<210> 29  
 <211> 373  
 <212> DNA  
 <213> Homo sapien

<400> 29  
 acttgagatc cacagtcacg tgaactttgc cggctctctt acatctgccc acttcatttt 60  
 cattctttcc ttccacaca atggtttttc caatgtgcaa gaatgatttc tcgacaaatt 120  
 cccggacact atggacctcc ccagtagcta taacgaaagc cttccgggtc tcattctgca 180

acatcaacca	catagcctcc	acatagtcct	tggcatggcc	ccaatctcgt	ttggcatcca	240
gatttcccaa	actgaaacat	tccagttgtc	caaggtaa	cttagctact	gaccggctaa	300
tttttcgagt	aacgaaatta	gcttctcttc	tgggactctc	atgattgaag	agaatgccgt	360
cactgcaaag	aga					373

&lt;210&gt; 30

&lt;211&gt; 485

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 30

aaaactacga	ctcagcatac	atthttccac	atacatthtt	acattgtacc	ttaggactca	60
gtcatctcca	cttaaatga	tgacacaagc	agctaataac	catttctggg	tttctgccta	120
acccctaat	tgtctgttaa	agccaattct	ctgggtgtcc	cagtgaagg	tggtttttt	180
tctttccaca	ttggcacatt	cacttctccc	actcttggca	tgtaagaaat	aagcatttac	240
ataattggaa	aaatctggat	ttctgatgcc	aaagggttaa	agcttcttgg	atttcatttc	300
attgatatac	agccactatt	ttatttttga	tcagtggcct	ttggggccact	gttcagggtta	360
ctgaccatca	gtgtcagcat	tagggttttg	gtttttgttt	cttttgggtc	tttctttttt	420
ggcacatgtg	aatcttgttt	tgtgtaaaaa	gaaattactt	tctcttgttc	tctgatgatg	480
ggttt						485

&lt;210&gt; 31

&lt;211&gt; 342

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 31

acacattaa	catccccagt	tcccctcgca	cacccctttt	cccagccact	agtaaccatc	60
cttctactct	ctatatccat	gagttcaatt	gttttgactt	ttagatcccg	caaataattg	120
agaacatgca	atgtttgtct	gtttctggct	tatgtcactt	aatatagtga	cctctagttc	180
catccatgac	tccttaactg	cccctgaatt	tttgacacta	ttatttttaa	gtattttgga	240
aaactcacac	ctgttctcat	ttttaaacct	taataataac	aatttcttac	taagctaata	300
aaacttcccc	ttatattatt	tgtaatgtgt	gcataacata	gt		342

&lt;210&gt; 32

&lt;211&gt; 331

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 32

acagtatgtg	gcatttccag	gtatgactga	gtgtgagaga	catgtcagag	gctcttcagt	60
gatttcttgc	tattgaccga	tgcttcaact	tgccaaaaga	gaaaaaaat	gttgggtttt	120
gtaattaaat	tatttatata	tttttgaaac	ccgaattgaa	aatgttgcag	gcaacgggct	180
acagctttat	tagtggttct	ctaactgtgg	tctccttggg	ccaagcaatt	tctttaaagg	240
aaaagttgat	tatgtatgtg	gagtgccagg	accactgcct	tgaaagcaag	tgtgattttt	300
atthttaata	ttattttatt	tgtgtctgtg	t			331

&lt;210&gt; 33

&lt;211&gt; 381

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 33

acactgttgg	tgttatatgg	ggatgggggt	ctcggtaatt	ttgtttatta	tttatgttta	60
ttattatggt	ttatcattaa	ttattcaata	aatttttatt	taaaaagtca	ccctacttag	120
aaatcttctg	tgggggtggg	agggacaaaa	gattacaaac	caaaactcag	gagatggtaa	180
cactggaatt	gataaaatca	cctgggatta	gttgtataac	tctgaaccac	caaacctctg	240
ttatcaagcc	ttgtacagtt	catggctgtc	cagaaagatt	tacagttatt	tttctgagaa	300

aggatccatg ggctttaaga acttcagaac ttttaagaact tcagaagttc ttaagttgct 360  
gaagctcaag taacgaagtt g 381

<210> 34  
<211> 315  
<212> DNA  
<213> Homo sapien

<400> 34  
acgaaactgt atgattaagc aacacaagac accttttgta tttaaaacct tgatttaaaa 60  
tatcacccct tgaggctttt ttttagtaaa tccttattta tatatcagtt ataattattc 120  
cactcaatat gtgatttttg tgaagttacc tcttacattt tcccagtaat ttgtggagga 180  
ctttgaataa tggaaatctat attggaatct gtatcagaaa gattctagct attattttct 240  
ttaaagaatg ctgggtgttg catttctgga ccctccactt caatctgaga agacaatatg 300  
tttctaaaaa ttggt 315

<210> 35  
<211> 567  
<212> DNA  
<213> Homo sapien

<220>  
<221> misc\_feature  
<222> (1)...(567)  
<223> n = A,T,C or G

<400> 35  
tacttcttaa aanacatata acacaatgtg gtagtagtag gtgtaaggaa ggtaagtttt 60  
ttcatagtgg tatgcaaaca tatcattgaa atattacata gatataaaga cttagggaat 120  
aaaaatagca gcaacaaata cttgatagat ttatcctact tgggagaaat attttgtagc 180  
agagtattta gtatacttag aagttgattt agcaattagg ctttaatgac cttacaaagt 240  
gaacataact gaacacaagt attttttcaa tgcaagatga ggatgaaaat tttacatttc 300  
aaccatctg gctaaagtta agacttagca aaaattaaaa tgttgccttt gtccaagtat 360  
agattaaggc acaaaacata tttgggtgtg taatttgaag ttttgactg aaatatcttt 420  
gcaagtatcc acataaaatt ctgtaatgcc ttataattat attctaataa ttatgcatta 480  
tactaagaca ccattaagaa cagttgange actacactaa atcaaaccat aaatgaggaa 540  
aaaactttta atggtctttt ctagaag 567

<210> 36  
<211> 265  
<212> DNA  
<213> Homo sapien

<400> 36  
acaagtgggtg gccacagaag taggggggtc ttccttaagc tctgtgtcag agttccacct 60  
gatccttatg gatgtgaatg acaaccctcc caggctagcc aaggactaca cgggcttggt 120  
cttctgccat cccctcagtg cacctggaag tctcattttc gaggctactg atgatgatca 180  
gcacttattt cgggggtcccc attttacatt ttccctcggc agtggaagct tacaaaacga 240  
ctgggaagtt tccaaaatca atggt 265

<210> 37  
<211> 476  
<212> DNA  
<213> Homo sapien

<400> 37  
actgtatgtg ttttggttaat tctataaagg tatctgttag atattaaagg tgagaattag 60  
ggcaggttaa tcaaaaatgg ggaaggggaa atggttaacca aaaagtaacc ccatggtgaa 120

gtttatatga	gtatatgtga	atatagagct	aggaaaaaaa	gcccccccaa	ataccttttt	180
aaccocctctg	attgggctatt	attactatat	ttattattat	ttattgaaac	cttagggaag	240
attgaagatt	catcccatac	ttctatatac	catgcttaaa	aatcacgtca	ttctttaaac	300
aaaaatactc	aagatcattt	atatttattt	ggagagaaaa	ctgtccta	ttagaatttc	360
cctcaaactc	gagggacttt	taagaaatgc	taacagattt	ttctggagga	aatttagaca	420
aaacaatg	atcttagtaga	atatttcagt	atttaagtgg	aatttcagta	tactgt	476

<210> 38  
 <211> 424  
 <212> DNA  
 <213> Homo sapien

<220>  
 <221> misc\_feature  
 <222> (1)...(424)  
 <223> n = A,T,C or G

<400> 38						
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tnantggaaa	cctgattctg	gcagctcatt	tatcttggtt	tcctcatttg	taaggctggt	120
cagttggact	gatcatctct	gagggccttg	aagccctaac	aagtctatca	tgatcccaga	180
tgtaaaatat	atatatgtgt	atatatataa	tttcagctga	gaagtgtg	ttcacaccaa	240
gtctactttt	tgcaagttac	tgggtttctg	tcttcacat	cttctgaaaa	gtctgcttct	300
gttggttcag	tttctggggt	catctgagta	gagagattct	gaaacagaca	ctgatgttaa	360
tttgggggac	tacttttctc	atgcaaacag	gggagctcct	ancaatcctg	agaggngctg	420
catc						424

<210> 39  
 <211> 493  
 <212> DNA  
 <213> Homo sapien

<400> 39						
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actcttcaag	ttcaaccact	gttaagacct	cctattgagt	tttccaggtc	ctcagatggg	240
aattgcacag	taggatgtgg	aacctgttta	atgttatgag	gacgtgtctg	ttatttccta	300
atcaaaaagg	tctcaccaca	taccatgtgg	atgcagcacc	tctcaggatt	gctaggagct	360
cccctgtttg	catgagaaaa	gtagtcccc	aaattaacat	cagtgtctgt	ttcagaatct	420
ctctactcag	atgaccccag	aaactgaacc	aacagaaagc	agacttttca	gaagatgggt	480
aagacagaaa	ccc					493

<210> 40  
 <211> 464  
 <212> DNA  
 <213> Homo sapien

<220>  
 <221> misc\_feature  
 <222> (1)...(464)  
 <223> n = A,T,C or G

<400> 40						
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ttcccctaca	tctggacatt	ttgaaatagt	ctttgggtatt	actagttatt	gtgctttgaa	120
acagaaactt	gcagaatttc	tgtagtagtg	ctacataaag	atataaataa	gaaaaatgca	180
cttggaaata	gttacattta	gctgcttttg	cataattttc	aaaaactaca	gtgtatgcct	240

agtcacagtt	ttatgagaaa	gaatatttcc	tttttcaact	taattttaag	gaacacttaa	300
tcattttggc	taagtatcca	tttttggagt	ggatctgatg	agttgcatga	cactaaactt	360
ggatgctctc	catttgctga	aaggcacatt	tttaagaatg	gattgnatag	aagttgatcc	420
ttctggatct	cccatatctg	ctctccagtg	acaactgnct	tgtg		464

<210> 41  
 <211> 557  
 <212> DNA  
 <213> Homo sapien

<220>  
 <221> misc\_feature  
 <222> (1)...(557)  
 <223> n = A,T,C or G

<400> 41						
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gttctgagtt	ggtgaccana	aagttgcatg	tagngctggc	acttacttaa	taactattca	120
tgatattggt	aataacttgt	tataggattg	tattcccaat	tacagtctct	aanattgtaa	180
ttgatattat	ctganaggna	gngngacaac	tttcttttgt	tgttacatta	agccgaaaac	240
ataatactaa	tagacaacta	acagtttgct	tatcaggcac	atcaactaag	gcacctcccc	300
ccatgctaag	tttctcctgg	atatatggaa	gttgattggt	tcccagttna	aaaacttgaa	360
ctaatatctc	ctaaaaaaat	ctgagtccat	attgttttta	ttttacttag	ctanaatctc	420
atagcangtt	aaagtcatat	ccttatcccc	actaaaaata	actatgtnta	tgtgagagga	480
atatagtatg	tgggagctgt	attaaatact	attacaggtg	ttacagaatc	tttaaataaa	540
tggacatgga	ccaactt					557

<210> 42  
 <211> 255  
 <212> DNA  
 <213> Homo sapien

<400> 42						
actatcaggc	tttgtgctga	tttctgaac	aaactgcatt	atattatgaa	aacaaaagga	60
aaagaagaaa	taataaaaaac	tatactccca	tatttcactt	acagtgtttg	agttcctgga	120
aggacctata	taatggaggc	agcattcaaa	caagaaatta	tgccaatcaa	ctgtcaaatt	180
ttcactataa	ttttcctaaa	aaggcgtttt	tcccccaata	tctattaatc	tcaaagaaac	240
ataagttgtg	aatgc					255

<210> 43  
 <211> 349  
 <212> DNA  
 <213> Homo sapien

<400> 43						
actccagcag	atttaatat	ggcatccatc	atctagtcaa	acctctcaca	tgttcttcaa	60
atcaatcaaa	tttgggattc	tcaacatttt	ctgtgtcaat	aaaagggtgtg	gaattagtag	120
attcgatgaa	gacctgtttt	tccttgccac	attggacttc	cagacgccat	ttggattggg	180
tttagaagat	ggggaaattt	agaagacgtt	tcttggcctg	agtcctctaa	gagtagagat	240
gcagaagaga	gagtgagacc	acgaagagac	tggtgtttga	ctgcagggca	ccaccagccg	300
ccttgggtgt	ggcattagtt	ggatttgggg	ccaaccaga	gttggaagt		349

<210> 44  
 <211> 483  
 <212> DNA  
 <213> Homo sapien

<400> 44

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cgttttttgc	tgctatggtc	ttagcctgta	gacatgctgc	tagtatcaga	ggggcagtag	180
agcttggaca	gaaagaaaag	aaacttggtg	ttaggtaatt	gactatgcac	tagtatttca	240
gactttttta	ttttatata	atacattttt	tttccctctg	caatacattt	gaaaacttgt	300
ttgggagact	ctgcattttt	tattgtgggt	ttttgttat	tgttggttta	tacaagcatg	360
cgttgcaact	cttttttggg	agatgtgtgt	tgttgatgtt	ctatgttttg	ttttgagtgt	420
agcctgactg	ttttataatt	tgggagttct	gcatttgatc	cgcatccctt	gtggtttcta	480
agt						483

&lt;210&gt; 45

&lt;211&gt; 281

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 45

acatcgagaa	tccacgcccg	gggaccagta	ggacttgagg	gactgcttac	tactaagtgg	60
ctgctgcgag	ggaaggacca	cgtggtctca	gatttctcag	agcatggaag	tttaaaatat	120
cttcatgaga	acctccctat	tcctcagaga	aacaccaact	gaaaagagcc	aggaaaaccc	180
gggaattttc	caaaagggtc	tcacgttaaa	cttgtcttat	ctcaggagag	agcccgtctt	240
tgtctcccag	ttcctggtag	ggtctgcctg	ttggaaagtg	t		281

&lt;210&gt; 46

&lt;211&gt; 587

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 46

acagcccggc	ctcccttgat	gcatttggcg	cgcttctgaa	aagttgtgtg	taaaggaaga	60
atthgccatc	aagccatttc	ccccctttgt	ttctaaaaat	atttcagaga	tgtgtgctcc	120
tggagggaag	aagaaatagc	gcctcaacag	attaaaaaac	aaaagtcaca	cttaaggatc	180
cttctagtca	catcagcagt	gttctgcctt	tatgtagtag	ttgggcatat	aatccttcca	240
cacagcccct	gcaggggaaag	gctaattctta	cggataatcc	acgtgagatt	tccacacaag	300
agaaaagcac	acgcatagtg	aaatgtcagt	cttttcagta	atgaggatac	ctttaaggca	360
ctcttggact	ctcggcaacc	acaacataat	agttgaaaaga	tcaagattgg	ctccacgaaa	420
gtgatacgga	ggttaggatg	ctacttgctg	caaacaagcc	ctactttggc	caacatcctg	480
cttatttctc	aaaaaagagg	gacagtgaag	acaaaaacga	cattgggaca	tgctgctcaa	540
ggtagttata	tatacgataa	gttgtatata	tgatcactgg	tagccta		587

&lt;210&gt; 47

&lt;211&gt; 317

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 47

gaggactctg	acagccataa	caggagtggc	acttcatggt	gcgaagtga	cactgtagtc	60
ttgtcgtttt	cccaaagaga	actccgtatg	ttctcttagg	ttgagtaacc	cactctgaat	120
tctggttaca	tgtgtttttc	tctccctcct	taaataaaga	gaggggttaa	acatgccttc	180
taaaagtagg	tggtttttgaa	gagaataaat	tcatcagata	acctcaagtc	acatgagaat	240
cttagtccat	ttacattgcc	ttggctagta	aaagccatct	atgtatatgt	cttacctcat	300
ctcctaaaag	gcagagt					317

&lt;210&gt; 48

&lt;211&gt; 512

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 48

acacttgat	ggcttttcac	cagtgtgagt	cctcaggaga	gcttttaaat	gagaagactt	60
ggtataaact	tttgtgcaac	cagggtaatc	gcagtagtgg	atgcgtcggt	tctccaaatc	120
ggggttactc	cttctattgt	atctgacagg	ttggatgttt	tgtgagttaa	ctggcagggt	180
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aggtggggtt	aaattctgga	gcattctctgc	ttgtctatct	ggacttccag	gctctgagct	300
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tgtgttaagg	cctgccatgg	cagctgacat	agaaacatta	agagtgtcca	ttgctgctgt	480
ctgatttgta	gaactgggca	tatctagatc	cg			512

<210> 49  
 <211> 454  
 <212> DNA  
 <213> Homo sapien

<220>  
 <221> misc\_feature  
 <222> (1)...(454)  
 <223> n = A,T,C or G

<400> 49						
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agagaatgca	gaaaatgagt	atcagacagc	aattagttaa	aactatcaaa	caatgtcaga	120
taccacattc	aaggccttgc	gccggcagct	tccagttacc	cgcaccaaaa	tcgactggaa	180
caagatactc	agctacaaga	ttggcaaaga	aatgcagaat	gcttaaaggc	tgatgtagg	240
attcttcagt	atgtggaaag	acaaggattc	aacgtgtggt	catatgataa	ataagtgtt	300
tataaacaag	agtgtatatt	tgctagggct	ttcaaagtta	accggttttc	tagcctcatg	360
gaatactgtt	gaacctatag	cgttgtcttg	attcttttgt	gttctctgcc	ttgtaatttt	420
ctgttactgc	tatatctacg	tgtaaatctt	tntt			454

<210> 50  
 <211> 374  
 <212> DNA  
 <213> Homo sapien

<400> 50						
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gccatcattg	tgataaatga	ggagtgttggg	ggctgttcct	gagaattgta	gataccacga	180
cacataatta	gttccaatgt	tgagggcgct	tccagagcag	gacatggaga	ccttctgtcc	240
tggggcccga	gagactgagg	gcggctgcgt	caagatggac	tgggcccagg	accctgtgca	300
gtgaatgaga	aggggtgagg	ggagagggga	gcaggtcatg	atgaagattg	tcccagatcc	360
tgcccttctgc	gctc					374

<210> 51  
 <211> 250  
 <212> DNA  
 <213> Homo sapien

<400> 51						
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gtaaattatc	tttctaattg	tctgtgaggc	caaacattta	tggtcagatt	gaaatttaaa	120
ttaatatcat	tcaaaaggaa	acaaaaaatg	ttgagtttta	aaaatcagga	ttgacttttt	180
tctccaaaaa	catacattta	tgggcaaatt	gtgttcttta	tcacttccga	gcaaatactc	240
agatttaaaa						250

<210> 52  
 <211> 351



&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 52

acgaaaggggt	ttgtaccaat	attcactacg	tattatgcag	tatttatatc	ttttgtatgt	60
aaaacttttaa	ctgatttctg	tcattcatca	atgagtagaa	gtaaatacat	tatagttgat	120
tttgctaaat	cttaatttaa	aagcctcatt	ttcctagaaa	tctaattatt	cagttattca	180
tgacaatatt	tttttaaaag	taagaaattc	tgagttgtct	tcttgagct	gtaggtcttg	240
aagcagcaac	gtctttcagg	ggttggagac	agaaacccat	tctccaatct	cagtagtttt	300
ttcgaaggc	tgtgatcatt	tattgatcgt	gatatgactt	gttactaggg	t	351

&lt;210&gt; 53

&lt;211&gt; 546

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 53

acatggacat	tctgcaaacc	cagctgtcac	atttttcttg	caactccttt	tgcaaaagca	60
gactaaaatg	ttttaaaatg	tgaaaaaaca	ttattttttc	aaagcaagaa	aataattttac	120
tgccctctta	cataatgtat	ttataaagtt	tttccagata	aactaatcaa	ataaattaga	180
ataatgtgac	aacattacaa	atttaatttg	ttagctgcat	tccttctgat	gttaccacga	240
tagaatgtta	ctgatgattc	agggctattt	ctgaagtctg	tatgttgetg	ctgtccccag	300
tgatgggtgga	cttatctttg	ccttacctga	tcacaaatta	tgttggggaa	aataaagatt	360
taatatttct	ttaaatagaa	aaagaatttg	gttttgctcg	tttaagagca	atgagaaaat	420
gatggaatgt	tgactgtgtt	tggcacacag	gacacggacc	ttcatggaag	tccttgctct	480
gcgtggcatc	tgtcagcttt	tcacctttca	ttcttattct	tcacttttgc	tgctgagcct	540
agctgt						546

&lt;210&gt; 54

&lt;211&gt; 631

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (1)...(631)

&lt;223&gt; n = A,T,C or G

&lt;400&gt; 54

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atctcaatac	acgatctctg	aagttcctaa	aattctggca	ctaattctaa	tgtgaactta	120
gtagcaaaag	accagaaaat	agtaagccct	tgacctaaaa	actaactgat	ttgtatgata	180
ttcatgcaga	aacaatgatg	aaatggagtc	aagttttcta	gtgtcattgt	tatcaaaata	240
actgtcaaaa	tagtaagttt	gaaacttaaa	tgagcacaaa	ataaaatttt	gttttctaac	300
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acacaagata	gtttaaatac	accttttaggt	gtagggggagg	ggagaagcgc	ctctttttct	480
aatgcagctg	ttttaatttg	aagcttttgc	acaaaatcag	atagaaacat	taatgcctaa	540
ctcataatga	cccttgatta	cttgtaattt	tggaactagaa	ataatgtggc	tttgaacatg	600
ccagtgttag	accatactga	cttaaaaaaa	t			631

&lt;210&gt; 55

&lt;211&gt; 408

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 55

accaatatat	ccccagaaag	aattgcaatt	taccaaggtt	ttcacgtgtt	ttgagagaaa	60
------------	------------	------------	------------	------------	------------	----

tcttactgaa	agactagtga	tgtccatttt	ccagtaaata	ctgagcgaaa	aacaattttt	120
atacccaat	ctgagggtata	aacttgcttt	ttgtgggatc	acaactgctg	taaattagac	180
aattgtagca	acaatccaag	acaataacag	aatgcctatg	acagtctgcc	atattctggt	240
gagtgtctat	caaagctcat	catgattttt	tgtgagatct	tccccgtaat	tggtagcttg	300
gcttccaaca	aacatgttcc	agttctccaa	tatttcctct	ttagttagct	tctcatcctt	360
gtttttgtct	gattcatata	ccagatgcct	ggcctcagcc	tgtgctg		408

&lt;210&gt; 56

&lt;211&gt; 567

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 56

actgtgggtc	gaagtaatgg	atacggacgt	aaccatcttc	gccgccgctg	ctgtagctct	60
tgccatcagg	atggaaggca	acactgttga	taggtccaaa	gtgacccttg	actcttccaa	120
actcttcttc	aaaggccaaa	tggaagaacc	tggcctcaaa	cttgccaatc	ctgggtggagg	180
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tggaataactg	gatgtcggtg	atctgccggg	agtgtcctt	aacattcacc	aacacctctc	420
cagacttggc	actatactgg	ttgagctctc	cactctcatg	gccagcgatg	atgcactccc	480
ccaggggtcc	ccaaacagca	ctggtgattt	tagagtcatt	gcaagggatc	ttcatgtagg	540
gtcattgggt	gtcaatctgg	ctcggt				567

&lt;210&gt; 57

&lt;211&gt; 411

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 57

acccttcctt	gtccgaagga	gctgaccagt	attgatgaga	gagtccaggc	agctcctgaa	60
gttcagctgg	tagtttggtc	tctgaacatt	tgggtctctg	aaggcacagt	atatctgggg	120
cttcttctct	taccaaatct	aatcctttct	tcttaatcca	ggctcgaagc	ccatccacat	180
tccaagagca	gatcttgagt	gtggcaggtt	tgccactggg	tgaggttttc	tgatctgggg	240
ggtcctcata	cagggctggg	ccctctcctg	ctgcctcttt	gtcatttttc	tttgcgccg	300
tcttactctt	cttggcctct	ggctctgtcc	tgagctcatc	cccgtcttcc	gccaccgctc	360
cctttttccc	acgcttcggc	attcccgtta	cgaacgcctt	tgggcagctg	t	411

&lt;210&gt; 58

&lt;211&gt; 589

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (1)...(589)

&lt;223&gt; n = A,T,C or G

&lt;400&gt; 58

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aaggacgaaa	ggtcagccct	aaagacaggg	tgttttgtta	ttatggtaat	tacaccttca	120
taccttctat	aatattcatt	gacagacggt	gacatcaaca	ggtgtagttt	atcatgttct	180
gtgtagagaa	ctaaactacc	ctactgtatt	tgccatgccc	ccaattccaa	gaaaacggca	240
aaaaatttag	ccatcccatt	cctcatcaca	aagatcttaa	ctgcaccctt	gcaacacaag	300
acttttccaa	taggacaaaa	cttcaaacag	cattgtatac	caaagtattg	cggtacaaaa	360
ttaaatttac	aggaacacaa	tactgaagca	ctccactggt	gctgtaaaaa	ctgctggaaa	420
cagaatctgt	caactggcca	aattttatcc	ttaattatta	tccaaacagc	cgctcctctc	480
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caactctttca ccattttctcc caccctaaga ggtacctgcc cnggcggnc

589

<210> 59

<211> 440

<212> DNA

<213> Homo sapien

<400> 59

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gagtataata	gtcattctcc	ttgatatggt	tgccctgtatc	tgtgctccct	ccaatccgga	120
ccatccaaag	aaacttggtg	atatcatcag	aggaataccc	agtgaggcct	cacaaaaatga	180
ccagcacata	gctgacatcg	agctccctca	tgatctcata	ggctttttcc	tctgtggacg	240
ccattgcctg	ccctactcga	gaaatatggg	tattattcca	tgtgttattg	tccactaaaa	300
ttgttcgggt	tgccatagct	gtaatctgat	agccataatc	ccaccaggac	atgaccttcg	360
catcctctgg	agtattatga	cgaagccaat	aatatgcttc	tcggaagtca	tcaaatatga	420
tcctactgcc	atccccacca					440

<210> 60

<211> 417

<212> DNA

<213> Homo sapien

<400> 60

acctggaaga	tcaagatcta	cagctgccta	ttccacatc	tttcaatcca	tctggctcct	60
taaatagggg	aaaaagccct	tatttgggtg	agaagcattt	ccaaaatgaa	gttacaggtt	120
ctattaaaac	ttactgtcac	atcaactggt	aaaatagggc	cttttgggtt	ttgttatttc	180
accttaatat	caccagaatt	cctgtaattc	cacaattgtg	attttactat	gtagaagata	240
attcagttct	agtctattgc	tttagatgta	aaaacagctg	aaaacccaaa	gtggattaga	300
attgctgaag	gatttccctg	ccgttgtttg	atacaatcta	ttctcttgat	tcttgatagg	360
tgcatagaaa	gcctaactta	aaattctttc	tacaggaaca	tgtctgattt	caggagt	417

<210> 61

<211> 354

<212> DNA

<213> Homo sapien

<400> 61

acctcctgtg	ttgcagagtt	tctttatcca	catccaccca	accagcagca	tcagccacag	60
gactgggtctt	gaggacatct	ggtgggctca	ttggagggtg	gacatgaagg	atttcatatg	120
aaatcacttg	ggtctctcct	ggtttgtcca	ggttctcaaa	tacagcctct	tgtttatcgg	180
ctcggacttc	aatgagggtt	ttctttagt	taacagtgag	gttccgctcc	tggtatgatct	240
cctgcagggc	atctgcatac	ttcttaaccc	cgaaaatggc	tccaagagaa	gtgttgaaaa	300
tgatattggc	cttggaatgc	ttccctgtct	tcctgaagta	ggcttctgat	aagt	354

<210> 62

<211> 205

<212> DNA

<213> Homo sapien

<400> 62

accccccttc	acttcgtctc	ccctagctcc	tagaagcaac	cactgatgtg	atttctacca	60
aatccagttt	tggtcctact	aaatatactc	ttttgagact	ggcctctttt	actcaccata	120
atgcctttgt	aattcatcca	tgctgtttgt	tgtatcagca	gtttgttcc	tttcattgct	180
gagtagtatt	ctattgtaga	gatgt				205

<210> 63

<211> 325

<212> DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 63

acacacgggt	tccggatcaa	tgctcgggcc	aacgccactg	cctgtcgtcg	accccttgac	60
agctggctcc	cagcctcgtc	tacctctgtg	tcatagccct	gagggagtcc	agagatgaaa	120
ctatggggccc	cagacttttac	tgacagcagct	gtgatttcct	ccatagttgg	cttctgggtc	180
aggccatagg	caatatatttc	ttgaagactt	cttccaaata	cctgtggctc	ttgtccact	240
gcagccacct	gcctgtgcag	gtagcgggtc	tcatatggg	gaaggggctt	cccatccaac	300
agcagctgtc	ccccggtggg	ctggt				325

&lt;210&gt; 64

&lt;211&gt; 599

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (1)...(599)

&lt;223&gt; n = A,T,C or G

&lt;400&gt; 64

actttgatgt	ttgaacaacc	ttttcttgat	cacttcttcg	caataaaaat	atgacatatg	60
tagtaaacct	taaaaaattt	cgtgtaactt	tatggctcta	cgctggaatt	cttctgaagt	120
gagtaatcat	cacaatcatc	tttagtatat	aatggatcaa	aatgacacga	ttgcaaatat	180
tgataacaca	cagttataaa	aggtgaaatt	ctattgggaa	cacatctctt	agtgagatag	240
atggggctga	cccaccaatt	aattcattta	tctggatgaa	tagttcctac	tggtagatta	300
acaggggtca	ttttcaattc	tggtgttttc	acagatacaa	gtgctgagaa	atgggtttac	360
ataaatagggt	gagaatgcta	gtagttttgt	tgtaagcatg	tcaatcaatc	gtttgggttc	420
tttccgagtt	gcatgccaaa	aaccaaatag	tgttccttca	tcagctgaca	attcatgggc	480
caccattaat	tttggtgaaa	gcaaagaact	ggaaaccatc	tgacttgaaa	agaatttggt	540
atcctgggtat	tagaggcatt	cactttctct	agnactttt	aattatacta	attactctc	599

&lt;210&gt; 65

&lt;211&gt; 373

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 65

acattaaagt	gtgatacttg	gttttgaaaa	cattcaaaca	gtctctgtgg	aaatctgaga	60
gaaattggcg	gagagctgcc	gtgggtgcatt	cctcctgtag	tgcttcaagc	taatgcttca	120
tcctctctaa	taacttttga	tagacagggg	ctagtcgcac	agacctctgg	gaagccctgg	180
aaaacgctga	tgcttggttg	aagatctcaa	gcgcagagtc	tgcaagttca	tcccctcttt	240
cctgaggtct	gttggtgga	ggctgcagaa	cattgggtgat	gacatggacc	acgccatttg	300
tggccatgat	gtcaggctcg	gcaacaggct	ccttggtgac	actcaccaca	ttgtttttca	360
agctgacttc	cag					373

&lt;210&gt; 66

&lt;211&gt; 520

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 66

acgtgagcca	gtcatccata	cactaaggcc	tagttgagaa	aaacctttga	ttcaggatgg	60
ctgggttact	aaccttgaaa	tgtaagagat	ctggttttga	atgtaaaagt	tgcaacacac	120
aaacggaagt	cttaaaaact	ttttgctctg	gtcagttaca	gggtgatccc	caataatctg	180
tttttggttt	tctgatggaa	ataatagaat	taggggaaat	caaactctgg	tggtaggtgt	240
ctacagtatt	agaagagggt	ataagggcac	tgtttaacac	taagttctaa	tacttccaga	300
aactgtgcat	tccagatcta	catactaaat	gctcttatca	ttttgaaatg	ggctcttgat	360

taatagaccc	atatttttta	gtggcttcta	tggtgtatat	ttgtctaaaa	tgaaagctct	420
tttgcgttct	aaaactacaa	tatatgtcat	cttattttcc	ctgagtatcc	aagtatatgtg	480
cagattctat	gtaaaactac	taaatgacac	tggaatatgt			520

&lt;210&gt; 67

&lt;211&gt; 241

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 67

acagagatgg	agaacgaatt	tgtcctcatc	aagaaggatg	tggaatgaagc	ttacatgaac	60
aaggtagagc	tgaggtctcg	cctggaaggg	ctgaccgacg	agatcaactt	cctcaggcag	120
ctgtatgaag	aggagatccg	ggagctgcag	tcccagatct	cggacacatc	tggtgtgctg	180
tccatggaca	acagccgctc	cctggacatg	gacagcatca	ttgctgaggt	caaggcacag	240
t						241

&lt;210&gt; 68

&lt;211&gt; 487

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 68

actttgaggg	attggtgggc	ttgggcccct	cctggcccag	gagatgtaga	atacgggtgg	60
ccagcactgt	gaactcgag	tcctcgatga	actcgcacag	atgtgacagc	cctgtctcct	120
tgctctctga	gttctcttca	atgatgctga	tgatgcagtc	cacgatagcg	cgcttatact	180
caaagccacc	ctcttcccgc	agcatggtga	acaggaagtt	cataaggacg	gcgtgtttgc	240
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agattttctga	catgaaggag	gagatctgct	tcattgagggc	gtcgatgctg	ctctcgctgc	360
ccgtcttaag	gagggtgggtg	atggccagcg	tggcaatgct	gcggtttgaa	tctgtgacca	420
ggttctccag	atccagatta	caagctgtca	cagctgacgg	atgcttcatg	gcaaccttat	480
tgagggt						487

&lt;210&gt; 69

&lt;211&gt; 415

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 69

actagcttca	agaagctttt	ggtcagctac	atttaaaggc	acaatagggc	ctttggattc	60
tttgtgtgta	attggttttt	cactgagtgg	tttgaagta	tctaaatcgg	actttttact	120
atattccaca	cttactacca	catccttggt	gccaggagat	ttctcttggtg	atgacaataa	180
ttctttctgt	ccttgaagat	gagatatatc	cagaccttct	tttaggcgaa	taaccactac	240
tccatattgt	atgtcaaaaag	catcatgaaa	taagtgttata	tacatatcca	catccctcat	300
atctgcttgc	aaccaatctt	tcttaaatcc	aaggacaagt	gtgtttggct	tcatacgacc	360
aagaccagca	gcctgcatca	aatactgtgc	accttctctc	aagtcatctg	catgt	415

&lt;210&gt; 70

&lt;211&gt; 535

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 70

acatcatgtc	ttataaggaa	gccattaagg	tcactccact	gccatgtatg	caactgctgt	60
gtggctcgat	atgatcaaca	ctgcctgtgg	actggacggg	gcataggttt	tggaaccat	120
cactattaca	tattcttctt	gtttttcctt	tccatgggat	gtggctggat	tatatatgga	180
tctttcatct	atttgtccag	tcattgtgcc	acaacattca	aagaagatgg	attatggact	240
tacctcaatc	agattgtggc	ctgttccctt	tgggttttat	atatcttgat	gtagcaact	300
ttccatttct	catggtcaac	atttttatta	ttaaatcaac	tctttcagat	tgcccttctg	360

ggcctgacct	cccatgagag	aatcagcctg	cagaagcaga	gcaagcatat	gaaacagacg	420
ttgtccctca	ggaagacacc	atacaatctt	ggattcatgc	agaacctggc	agatttcttt	480
cagtgtggct	gctttggctt	ggtgaagccc	tgtgtggtag	attggacatc	acagt	535

<210> 71  
 <211> 249  
 <212> DNA  
 <213> Homo sapien

<400> 71						
agcgggacga	ggatgacgag	gcctacggga	agccagtcaa	atacgacccc	tcctttcgag	60
gccccatcaa	gaacagaagc	tgacacagatg	tcattctgctg	cgctcctctt	ctgctcttca	120
ttctagggtta	catcgtggtg	gggattgtgg	cctgggtgta	tggagacccc	cggcaagtcc	180
tctaccccag	gaactctact	ggggcctact	gtggcatggg	ggagaacaaa	gataagccgt	240
atctcctgt						249

<210> 72  
 <211> 297  
 <212> DNA  
 <213> Homo sapien

<400> 72						
acacactgat	tgtgcggcca	gacaacacct	atgaggtgaa	gattgacaac	agccaggtgg	60
agtcgggctc	cttggaagac	gattgggact	tcctgccacc	caagaagata	aaggatcctg	120
atgcttcaaa	accggaagac	tgggatgagc	gggccaagat	cgatgatccc	acagactcca	180
agcctgagga	ctgggacaag	cccagacata	tccccgaccc	tgatgctaag	aagcccaggg	240
actgggatga	agagatggac	ggagagtggg	aacccccagt	gattcagaac	cctgagt	297

<210> 73  
 <211> 531  
 <212> DNA  
 <213> Homo sapien

<400> 73						
acttgtccca	ctcctgttca	gagggtcacat	gcttatccaa	aaactctgcc	atcccaatgc	60
ccattctccg	gcaaagtctg	gcaatcactg	tttggtatct	ctcagccaga	tttctaaact	120
caagggagat	cggttggaag	tcctccagca	cctggcgatc	cttctccttg	ctctccatga	180
accgccagtc	tggttggtaa	aggaaagagt	gaaagtgtg	taacagcggg	accttctttt	240
ccacactgat	ggatcatgtca	tcttcagtg	tgtccagagc	tcggagaacc	agataaaata	300
tgacacactgc	gttgcgcat	tccccatcca	gcgcctggat	aacagctgcg	aaactgcgac	360
tggtctgatt	gagatacttg	tagcaagttt	tcaggctgct	gctgagcgag	tcctgggtcca	420
tcttgggcat	caccttccgc	ttgccccga	tccggaagcg	caccaggttg	tagaactctt	480
cggggtggcc	aaggcatttc	acgaactoca	tcctggtgca	ggcggcggac	t	531

<210> 74  
 <211> 394  
 <212> DNA  
 <213> Homo sapien

<400> 74						
actaaaactt	acaataaata	tcagagaagc	cgtagtcttt	tacagcatcg	tctgcttaaa	60
agctaagttg	accaggtgca	taatttccca	tcagtctgtc	cttgtagtag	gcagggcaat	120
ttctgttttc	atgatcgga	tactcaaata	tatccaaaca	tctttttaaa	actttgattt	180
atagctccta	gaaagttatg	ttttttaata	gtcactctac	tctaatacagg	cctagctttg	240
ctcatttttg	agcctcacta	aaataacaga	tttcagtata	gccaaagtta	tcagaaagac	300
tcaaatggaa	tgattttaca	aatagaacac	tttaaaccag	gtcagtccta	tctttttgta	360
gctgaaggct	atcagtcata	acacaatttc	gcgt			394

<210> 75  
<211> 369  
<212> DNA  
<213> Homo sapien

<400> 75  
acattggtga tcggagtata gttggagcgc tttgtcatga tttccaggtt ggctttgtcc 60  
acagctatgt tggccaatgc accttgagcc tcaaagctgg caaatcgtcc aaattcttca 120  
agccgccaga ccgtctcctt ctttgccata tccacatgga aaatctcatc accatcaaag 180  
tcaaacataa actcgcctga ttggtcagga ttcagataga actcggcctg gatgatcaca 240  
tggtcttctt tgatagccca tgattcctga gcgctcatca gcacagctat gatgaaaaat 300  
cctagcacag ggactccact tatggccatt ttcttcttgg gcgctctgtt gggagtcagt 360  
agagctcgg 369

<210> 76  
<211> 384  
<212> DNA  
<213> Homo sapien

<400> 76  
acgactcggg gctcgccctg tccgcggcct tgcaggccac tcgagcccta atggtggtct 60  
ccctgggtgct gggcttcctg gccatgtttg tggccacgat gggcatgaag tgcacgcgct 120  
gtgggggaga cgacaaagtg aagaaggccc gtatagccat ggggtggaggc ataattttca 180  
tcgtggcagg tcttgccgccc ttggtagctt gctcctggta tggccatcag attgtcacag 240  
acttttataa ccctttgatc cctaccaaca ttaagtatga gtttggccct gccatcttta 300  
ttggctgggc aggggtctgcc ctagtcatcc tgggagggtc actgctctcc tgttcctgtc 360  
ctgggaatga gagcaaggct gggg 384

<210> 77  
<211> 291  
<212> DNA  
<213> Homo sapien

<400> 77  
acgtggcagc catggctccc ttcacaagct gtaggtcctg gtgggacagc tggctttggg 60  
gaagcttgtc tttctgggtg acccatggat gctgcagaac ctgcttagct gtgaggcgct 120  
ggtggggatc cacgtgtagc atcttggaca ccaggctcct ggctgtctct gaaactgtgt 180  
tccaaatttc cccactgagg gtaaaacttc cactgccgat ccgggttagg atttcctctg 240  
gtgtgtcact gggaccggtg gcaaattggag tatactctgc cagcatggtg t 291

<210> 78  
<211> 242  
<212> DNA  
<213> Homo sapien

<400> 78  
acctatattg ctaatgctag gatcaagata ccacatagcc agaacaagaa gttgaaggta 60  
aacatagaat attttataca ggcactcaca cctgccattt cggaaaagga ttaggaatcc 120  
agatgccgtg aatttaacta ttcgttacag gcttgtcctg caatatgctc tggagcaact 180  
tgctcgcaga gatttctgta tccacggaca tttaaatata gcaaaggcta tctccaggca 240  
ag 242

<210> 79  
<211> 449  
<212> DNA  
<213> Homo sapien

<220>

<221> misc\_feature  
 <222> (1)...(449)  
 <223> n = A,T,C or G

<400> 79  
 ngtacagaca aaactacaga cttagtctgg tggactggac taattacttg aagganttag 60  
 atagagnatt tgcactgctn aanagtcact atgagcaaaa taaaacaaat aagactcaaa 120  
 ctgctcaaag tgacgggttc ttggttgctc ctgctgagca cgctgtgtca atggagatgg 180  
 cctctgctga ctcagatgaa gacccaaggc ataagggttg gaaaacacct catttgacct 240  
 tgccagctga ccttcaaacc ctgcatttga accgaccaac attaatgcca gagagtaaac 300  
 ttgaatggaa taacgacatt ccagaagtta atcatttgaa ttctgaacac tggagaaaaa 360  
 ccgaaaaatg gacggggcat gaagagacta atcatctgga aaccgatttc agnggcgatg 420  
 gcatgacaga gctagagctc ggnccagcc 449

<210> 80  
 <211> 490  
 <212> DNA  
 <213> Homo sapien

<220>  
 <221> misc\_feature  
 <222> (1)...(490)  
 <223> n = A,T,C or G

<400> 80  
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 tctcaatgag agagtcctcg tcggttccca gcccttcctat ggaagctttt agctcanaag 120  
 cgtcatactg agcaggtgtc ttcaataggc ccaaaatcac cgtctccagg tggccagata 180  
 aggtgactt cagtgtgat gcaagttcct ttttggtcct tctctggtag gcgaaggcaa 240  
 tatctgtctc ctgtgcattg ctgcggntgg tcaaaatggt gacaatgggt acctcatcca 300  
 cacctttggt ctgatggct gtttcaatgt tcaaagcatc ccgctcagca tcaaagntag 360  
 tataggcttt gacagacca tatgacttg ggggtgtaga gtgatcacc tccaagctga 420  
 gcttgacacag gatttcgtga acagtagaca ttttgaagga agctgggccg tgcgccgaga 480  
 gctgagagcg 490

<210> 81  
 <211> 339  
 <212> DNA  
 <213> Homo sapien

<400> 81  
 acagtagtaa ctgatgtccc cttcttcctg gatgaatgag cagataaata ttgatgtcag 60  
 catccttgaa ccatatcaaa gtgagcagtg tttggctact gcttctattt gaaatggtgc 120  
 tgtgttttgg ttgtggtctg aagctttgaa gcgctactta gcatctcctt tcttccatgg 180  
 agctctcacg attcaaacat gacagatttg gtaaaatgct ggtagggttg agtcttcctt 240  
 gccccactc agtcatcttt gtatgaatcc catgatttgg gggttttttt cttttttttt 300  
 ataccagttt ttagctggtg tttatgaaga acagtgagt 339

<210> 82  
 <211> 239  
 <212> DNA  
 <213> Homo sapien

<400> 82  
 caagaacagc taaaatgaaa gccatcattc atcttactct tcttgctctc ctttctgtaa 60  
 acacagccac caaccaaggc aactcagctg atgctgtagc aaccacagaa actgcgacta 120  
 gtggtcctac agtagctgca gctgatacca ctgaaactaa tttccctgaa actgctagca 180  
 ccacagcaaa tacaccttct ttccaacag ctacttcacc tgctccccc ataattag 239



<210> 83  
<211> 528  
<212> DNA  
<213> Homo sapien

<400> 83  
acattcgtta ttttaaata acaagtttac aaagttttatt ttcattctata cgtaaggatg 60  
atTTTTTTTaa aactTTTTTtac atatttagtgg ttatgatcca atgtgtcatg agtgaattta 120  
actgtaagggt ggTTTTaaatc aaatatgcaa tgTTTTacttg aattgtattt ctatttagcag 180  
attttgacta tgTTTtacagg acggtTTTaaa ttaaggatta tcaggcatgt gagatctttc 240  
agttatcttt aaagtagatg tatattaagg gcttagattt aggatctaca tattctgggc 300  
attgaatagg cagtaactta caaataagtt ttgcttacct tttgttctag ggactagcac 360  
tgctatcaat ggaaagtatt tttactaat ctgttattaa gaaagtcata tttttgcatt 420  
tcagccaaaa taaagaccgc ctgtaataat ctgttagaaa cagataatac atgtctgaaa 480  
tccatatgtt tcatatgatc taaactgtat tttccaattt aaattaaa 528

<210> 84  
<211> 249  
<212> DNA  
<213> Homo sapien

<400> 84  
acactgaagc agaaccggaa acaccagga actgttcaga aatctcagaa gaaatctgct 60  
tctcttcgat ggaaagatat aattaacgat caaagagctc taagaaaatt gcaaagaagc 120  
cttaatgttc aagcttttaga aagatcagag caatttttct ctttcagtcc aaactaagac 180  
tctctgtatt taaatctctc tggggcaaga gggctagatt tcctcatttt gttatgagac 240  
tagattggt 249

<210> 85  
<211> 496  
<212> DNA  
<213> Homo sapien

<400> 85  
actggccctc ggtgctggca aaggtgtagt tccactggcc gagggaaatca agacatagtg 60  
gtccttctgc taagccaagg gctgccacaa tgacacagta gccagatcct gcaattccaa 120  
tgagagcagc caatacagaa gaaaacatcg cacatcgttt gccacagttt tcatggccac 180  
agcagccaca gcagtcaccc tgttccagcc caatgaagac aaatgctggc aggagcatca 240  
gcaggccacc tctacgatg ccagaaaaga accacacgaa gcggtgagg tggTTTTcg 300  
aggcatactt tgTTTTccca ttgggaaagt aaagcaaaat attaacgcg atgcacagga 360  
gggcgagccc caccagagaa tgtccgatgc atcgtgcaca cttcccatag cacatggtgg 420  
tctgctaggt tttctcccc ttctctttgt cttcagctca gtgatacccc aaattagatg 480  
aaagtgtgcc cttctg 496

<210> 86  
<211> 199  
<212> DNA  
<213> Homo sapien

<220>  
<221> misc\_feature  
<222> (1)...(199)  
<223> n = A,T,C or G

<400> 86  
acagaaagag taagataaaa acatttaata tnattaaatc taatttgcaa aaattggtat 60  
ctgacatttg ttgtgtgctc ttgcaaagag cgcataggac atttctgcag caatcaaaaa 120

ggtaaaatct ttttaaactc agatttcaag tttcctctaa tattccttct aatcctantc 180  
cctggaaata ctttcaagc 199

<210> 87  
<211> 436  
<212> DNA  
<213> Homo sapien

<400> 87  
aacgttttga tttcatgaag gtgtttctcaa atttaaagca cattttcagt aagaacaaaa 60  
atattttaatg tttttatctt agacttaact tgatacattt gcatattact atggaagtta 120  
ttcaccttgt ccctgttttt ctttaagata ttttaaaatc atagttatac tacagtcctt 180  
ttttaaatgt atcctgatac attgtaaaat attttaattt cattgtggaa aataatgttg 240  
gataaggaga tattttttcac tgttaacttt tagcccatgc attttcataa tttatttttt 300  
tcacttgctg ctttatatga catatgtgac atttgattat ttaacacttg atgtgatctg 360  
cataaaacca agttgcacaa ccctcctgct gaagataaaa ttgaggttaa agataaagat 420  
ttattttcat atttgt 436

<210> 88  
<211> 596  
<212> DNA  
<213> Homo sapien

<220>  
<221> misc\_feature  
<222> (1)...(596)  
<223> n = A,T,C or G

<400> 88  
acaaaagctg gtaatggacc aaagacttcc aaaatatatg tgtaatgacc tccagatttc 60  
tttatagtgtg ttcccaattc agcataagac aaagctccaa atagtgcag gacccacac 120  
accgtccaga tggtagaga catgcccacg ctgcccgtgt tctggagcac gcccttagga 180  
gagatgaaga ttctgtctcc aatgatggtg ccaatgataa tggagactcc cctcagtaaa 240  
gtgactttcc tcttcagctg cactttctcc tgcccagggt gctccttgtt gcccagggaa 300  
ggcagcctcc cgtaaactt tccctgcagg taacctcctt tggagatggt ggacacaaca 360  
ggctttctga ccatagtagg gacacacggg ggaaaaataa aacagaggga aagaaaacaa 420  
aactttcaac tttggtgtct cttggtgtta ctgatcgatg tcttctctctg ctttcagact 480  
gtctctctca gcgctatagt gttcacaggt gaaaactcaa aggtgtgctt tttncctcac 540  
agcgatctaa ttactactca gaaacacctg tgtatgcac gtgctctcaa ttcttc 596

<210> 89  
<211> 435  
<212> DNA  
<213> Homo sapien

<400> 89  
acacaagtca gtccaacagt tagtggttaat tactaataat atatgaaaac cctgccaaca 60  
caattgctgc tacatcacca atataattat taaccactgt cggaaaaaca cacataaatt 120  
caggtaagac taaaagctgt ctcacaaaaa gaaaaaagaa atccaatgga tccactaatg 180  
ctatcaaaag ggacatgcag gaatgtaaca tgacattttt agaaatgtgt. gtttctaaaa 240  
agaaaaaaa atacactaaa atgccagtgg actataattc attcaaaaaca tctttagtgt 300  
tccttcccaa agatcttgat ctgctcagta attgcttcac aagatctatc acagccatct 360  
tttgagcgt atggtaggac tggctcctct gtggtggttag gggcagctt tttgaagctt 420  
taagtatctg gtgggt 435

<210> 90  
<211> 344  
<212> DNA

<213> Homo sapien

<400> 90

actcagcgcc	agcatcgccc	cacttgattt	tggagggatc	tcgctcctgg	aagatgggtga	60
tgggatttcc	attgatgaca	agcttcccgt	tctcagcctt	gacggtgcca	tggaaatttgc	120
catgggtgga	atcatattgg	aacatgtaaa	ccatgtagtt	gagggtcaatg	aaggggtcat	180
tgatgggcaac	aatatccact	ttaccagagt	taaaagcagc	cctggtgacc	aggcgcccaa	240
tacgacccaaa	tccgttgact	ccgaccttca	ccttccccat	gggtgtctgag	cgatgtgggt	300
cggctggcga	cgcaaaagaa	gatgcggctg	actgtcgaac	agga		344

<210> 91

<211> 371

<212> DNA

<213> Homo sapien

<400> 91

agcaatgcaa	aggacatctc	caatcatgac	atttaagaca	attctttatt	tctctgacag	60
tgacttcttg	aagtgcacat	ataataaata	aatagaaaat	atatctttgt	tcatgggtgat	120
gcctacaaga	aatgtttaca	tacaaacact	ctatacatct	aactcccga	aaaggaccag	180
ctatttcggc	aacagaaaaa	agacaagcat	ttcagaggag	cgttgctttc	cttaaagacc	240
taactcactt	aagtcttaca	aacagaaaata	acaaggagga	caattttcta	agcaataaga	300
aaatttggtc	taccaagaaa	atgcctagat	attggctctt	ggtgaatggt	ttaggaaaga	360
aacttttatg	t					371

<210> 92

<211> 209

<212> DNA

<213> Homo sapien

<400> 92

acaacaaaag	atcaaaccga	tgtcccgatg	ttaacttttt	aacttaaaag	aatgccagaa	60
aaccagatc	aacactttcc	agctacgagc	cgccacaaa	ggccaccaa	aggccagtca	120
gactcgtgca	gatcttattt	tttaatatga	gtaaccacaa	tacacagctc	tttaaagctg	180
ttcatattct	tccccatta	aacaccagt				209

<210> 93

<211> 176

<212> DNA

<213> Homo sapien

<400> 93

actccctgtt	ttgagaaact	ttcttgaaga	acaccatagc	atgctgggtg	tagttgggtc	60
tcaccactcg	gacgaggtaa	ctcgttaatc	cagggttaact	cttaatgttg	cccagcgtga	120
actcgccggg	ctggcaacct	ggaacaaaag	tcttgatcca	gtagtcacac	ttcttt	176

<210> 94

<211> 494

<212> DNA

<213> Homo sapien

<220>

<221> misc\_feature

<222> (1)...(494)

<223> n = A,T,C or G

<400> 94

aaatggaaat	ttaantgaca	tcctanaggt	agagaaaccg	nggagatcnc	ttttctcaga	60
ctcaccaact	tttaatggga	tttcatgggg	tttggtttgtg	ctgatagggt	aaggggaggc	120

tgctttctgc	ccttctcccc	actcccatct	gatttactta	attcagtcct	agctgctgaa	180
atttggaag	gaccaaattg	ctttacagtt	tttttctttg	cgtagtatct	tgaaatcctg	240
gaaaattcta	tggaatagtt	ctgtatatag	ggcacaagta	aaggcattgt	ccaaagttta	300
tttatttatt	tattacccta	agaatgcttt	gccataacca	catttaattg	gaaaaacggc	360
annatcacag	atgtaaatta	nctcaccana	tttactgngc	ctgaactcat	tctcttcttg	420
ctatatgatt	tagcaagttc	tagaaggngt	ccaagacaat	aattacattg	gcacaatgta	480
tacttcagng	ctca					494

&lt;210&gt; 95

&lt;211&gt; 260

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 95

cgcggcgagg	tacgggcttt	ccatctagtt	gccagcttag	atctgggggt	ggtaaccac	60
tgactttgca	gtccattctg	cagagttttc	cttcttgaac	agtcagatct	ccaggagcct	120
gcaagaagtg	aggtctgaag	aatcgctcct	gaattgggtc	attttcgtct	ccactgtccc	180
ttgatctaga	acgaggcctt	ctgacatgag	gatggcctga	gggagaccgg	ggactccgac	240
ctctttgggt	gacagcctgt					260

&lt;210&gt; 96

&lt;211&gt; 438

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 96

accagttctt	gtttatatac	agtagtggtt	tgggcacacc	taaggtcgat	ctgtgttgta	60
tttaaaaaac	taatttcttt	atttggtggg	ccttctagac	aaacgaagg	gaccagagg	120
aaacccctg	acagatctct	ggatgatcct	ccttgaatcc	tgggcagttt	ggtctctcct	180
tgctgtgctc	ctgtggcact	aaactccttt	tgattgggtc	tttcttctct	tcccagctag	240
actaagcccc	tcatgggcag	gtaatgaaga	ttgaaaactt	ttttctgttc	tccagtgtga	300
gcacattcct	cctacatggt	agatgtgcaa	tagatgtttt	taaaattgga	gaatgaaaat	360
aaaagaagaa	aatcacattt	tcttatcaag	ttgtagcttg	gtatcataca	caattgcatt	420
ctgaggaatt	aaggtggg					438

&lt;210&gt; 97

&lt;211&gt; 454

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 97

gagtaattcc	cctccagcac	tagagaccgc	tcagtgtctt	tactagatga	actcagtaac	60
gccttgagct	gggttgattg	aggatgtgtg	aaaagctcac	agagctcgat	gcctgctgct	120
atttcacggc	aatgagcctt	tttctttcta	cactgaagat	tttcttctta	tttaatgtgg	180
tttatttttg	gtcagaaaat	aattgctctg	ttgaaaataa	tcctttgtca	gaaaagaagg	240
tagctaccac	atcattttga	aaggaccatg	agcaactata	agcaaagcca	taagaagtgg	300
tttgatcgat	atattagggg	tagctcttga	ttttgttaac	attaagataa	ggtgactttt	360
tccccctgct	tttaggatta	aaatcaaaga	tacttctata	tttttatcac	tatagatcat	420
agttattata	caatgtagtg	agtctgcgat	gggt			454

&lt;210&gt; 98

&lt;211&gt; 226

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (1)...(226)

<223> n = A,T,C or G

<400> 98  
 actaaatggt ggtctaggag cagctgggag natagcaccg ggcataatgtt ggaatggatg 60  
 aggtctggca ccctgagcag tccagcgagg acttggtctt agttgagcaa tttggctagg 120  
 aggatagtat gcagcacggt tctgagctctg tgggtagct gccatgaagt aacctgaag 180  
 aggtgctggc tggtaggggt tgattacagg gttggaaca gctcgt 226

<210> 99

<211> 333

<212> DNA

<213> Homo sapien

<400> 99  
 actcatctag acgttttaggt atttttcgtg gttgaggaag ctctctact aaattcttaa 60  
 gaatatcttc tggaatatac tcatctggaa aaagatgcaa cctttccatc attgttcttc 120  
 tgtgaagggt ttttggcagc atgccataaa tagctagttt tacaattgcc actggatccc 180  
 tcaggtgaag ctgagcagct gttacttgct taaatccacc tgggtagcca gtatgcgaag 240  
 agtatacttt ttgttcccat ttgtttccag aaaatgcaat gtgtcttggt ttcatataa 300  
 caacatgatc cccacagtca ctcagtgcgt ggt 333

<210> 100

<211> 417

<212> DNA

<213> Homo sapien

<400> 100  
 accgccacat cgctgacttg gctggcaact ctgaagtcac cctgccagtc ccggcggttca 60  
 atgtcatcaa tggcggttct catgctggca acaagctggc catgcaggag ttcattgatcc 120  
 tcccagtcgg tgcagcaaac ttcagggaag ccatgcgcac tggagcagag gtttaccaca 180  
 acctgaagaa tgtcatcaag gagaaatag ggaaagatgc caccaatgtg ggggatgaag 240  
 ggggtttgc tcccaacatc ctggagaata aagaaggcct ggagctgctg aagactgcta 300  
 ttgggaaagc tggctacact gataagggtg tcatcgcat ggacgtagcg gcctccgagt 360  
 tcttcaggtc tgggaagtat gacctggact tcaagtctcc cgatgacccc agcaggt 417

<210> 101

<211> 438

<212> DNA

<213> Homo sapien

<400> 101  
 acatatgttt tttaagtaag ttacttttac cattagaata aacctagaca ctacagggac 60  
 aactctgggg aacaggggcg tctgccttaa caacccttct ctaggttgag gaaggcaggt 120  
 atagttcact gaaggatgtg atgaggctgt agtaagtctt ctcatcatct gtaaatcctg 180  
 cgttgccttg tctcaccacc acagctacgt gcacatctgc tctctcagca gcactggcct 240  
 ctcgagtaac atctgtcaga aacaaaatgt tggtgggtga gcaccaatg ctgtctgcaa 300  
 tctttcggtg actttcactc tctactttgt gtccaatctt ggtatcaaag tgaccatcaa 360  
 caagctcaag aatatctccc tccgtagaat gccgaataa cagtttctgt gcctccacac 420  
 tccctgagga atagatgt 438

<210> 102

<211> 466

<212> DNA

<213> Homo sapien

<400> 102  
 acttaaaaag tggtttttct atcttcaaag tgctaaagaa acaagtattc aaaaagaaac 60  
 ttcaggtcgg tctacgaagt tctgactgac ttgaagtagt gaaataccaa gaatgcagtg 120

gacaaattta	aaaggccttc	attagaataa	agtatatctt	aactacattt	tgcaaagaaa	180
tgaagcaatg	gttgcacaaac	cagtcagggc	caagttagta	acatacaact	cagccatcag	240
cccacctctc	cctcaaaacta	aactaatcta	aatgtatttt	tcagaaaaatt	tcctccatac	300
tcctatgtatg	tgttacatac	atccaatcat	atccatattt	tggtatcattt	ttttctatat	360
tcatcagatt	attgggttaaa	atgcacagca	agtagaaatg	atccatttca	aaattcttaa	420
tatctagcgt	tctctgtaaa	acaaaagctg	acaacagttt	tattgt		466

&lt;210&gt; 103

&lt;211&gt; 500

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (1)...(500)

&lt;223&gt; n = A,T,C or G

&lt;400&gt; 103

nggtgcagcg	gagacagagg	cggaagctgc	agccctagag	gtcctggctg	aggtggcagg	60
catcttgga	cctgtaggcc	tcgaggagga	ggcagaactg	tcagccaaga	tcctggttga	120
gtttgtggtg	gactctcaga	agaaagacaa	gctgctctgc	agccagcttc	aggtagcgga	180
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cccctacagg	gagcacgtag	aggccatcaa	aattggcctc	accaaggccc	tgactcagat	360
ggaggaaagcc	cagaggaaaac	ggacacaaact	ccgggaagcc	tttgagcagc	tcagggccaa	420
gaaacaaatg	gcatgggaga	aacgcanagc	agtccanaac	cagtggcagc	tacaacagga	480
gaagcatctg	cagcatctgg					500

&lt;210&gt; 104

&lt;211&gt; 422

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 104

tggttctagg	agatatcaat	accaaaccac	agaaagaaaa	tattatagct	tttgaggaaa	60
tcatgaagtc	tgatggctc	aatgatttcc	tgaagatgat	aaagagcaag	atattgcaga	120
taaaatgaaa	gaagatgaac	catggcgaa	aacagataat	gagcttgaac	tttataagac	180
caagacatac	cggcagatca	ggttaaatga	gttattaaag	gaacattcaa	gcacagctaa	240
tattattgtc	atgagtctcc	cagttgcacg	aaaagggtgct	gtgtctagt	ctctctacat	300
ggcatggtta	gaagctctat	ctaaggacct	accaccaatc	ctcctagtcc	gtgggaatca	360
tcagagtgtc	cttaccttct	attcataaat	gttctataca	gtggacagcc	ctccagaatg	420
gt						422

&lt;210&gt; 105

&lt;211&gt; 326

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 105

acgaagttagg	tcaaaagtgg	ttgaccgtat	ttacagtctc	tacaaactta	cagtcataaa	60
acataaaaatg	aatactgaaa	gaatacttta	caagcaaaaag	aagaattctt	ctataagcat	120
tcctttttatc	ccagaaaacac	ctgtaaggac	cagaatagtt	tcaagactta	agccagattg	180
ggtttttgaga	agagataaca	tggaagaaat	cacaaatccc	ctgcaagcta	ttcaaatggt	240
gatggatacg	cttggcattc	cttattagta	aatgtaaaaca	ttttcagtat	gtatagtgtg	300
aagaaatatt	aaagccaatc	atgagt				326

&lt;210&gt; 106

&lt;211&gt; 543

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 106

acttgtaatt	agcacttggt	gaaagctgga	aggaagataa	ataacactaa	actatgctat	60
ttgatttttc	ttcttgaaag	agtaagggtt	acctgttaca	ttttcaagtt	aattcatgta	120
aaaaatgata	gtgattttga	tgtaatttat	ctottgtttg	aatctgtcat	tcaaaggcca	180
ataattttaag	ttgctatcag	ctgatattag	tagctttgca	accctgatag	agtaaataaa	240
ttttatgggt	gggtgccaaa	tactgctgtg	aatctatttg	tatagtatcc	atgaatgaat	300
ttatggaaat	agatattttg	gcagctcaat	ttatgcagag	attaaatgac	atcataatac	360
tggaatgaaa	cttgcataga	attctgatta	aatagtgggt	ctgtttcaca	tgtgcagttt	420
gaagtattta	aataaccact	cctttcacag	tttattttct	tctcaagcgt	tttcaagatc	480
tagcatgtgg	attttaaaag	atttgcctc	attaacaaga	ataacattta	aaggagattg	540
ttt						543

&lt;210&gt; 107

&lt;211&gt; 244

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 107

acaaaaatgg	ttataaaatg	gttgaagcaa	ctagaagcgt	gacaggtata	atacatataa	60
atacaaccaa	aattcaattc	aatgcaaagt	tgaatgacat	catattgcac	caaaatttat	120
tccatacaaa	agcacatgca	tcaagagttt	tcataagatg	aaaacaaaca	cacttacttc	180
atagcatctt	accacttact	tacacaaata	gcccataaac	accatctggc	attgtgattg	240
cagt						244

&lt;210&gt; 108

&lt;211&gt; 511

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 108

acttcatgtg	atttgtcaac	catagtttat	cagagattat	ggacttaatt	gattgggata	60
ttagtgcacat	caacttgaca	caagattaga	caaaaaattc	cttacaaaaa	tactgtgtaa	120
ctattttctca	aacttgtggg	atttttcaaa	agctcagtat	atgaatcatc	atactgtttg	180
aaatttgctaa	tgacagagta	agtaacacta	atattgggtca	ttgatcttcg	ttcatgaatt	240
agtctacaga	aaaaaaatgt	tctgtaaaat	tagtctgttg	aaaatgtttt	ccaaacaatg	300
ttacttttgaa	aattgagttt	atgtttgacc	taaatgggct	aaaattacat	tagataaact	360
aaaattctgt	ccgtgttaact	ataaattttg	tgaatgcatt	ttcctgggtg	ttgaaaaga	420
agggggggag	aattccaggt	gccttaatat	aaagtttgaa	gcttcatcca	ccaaagttaa	480
atagagctat	ttaaaaatgc	actttatttg	t			511

&lt;210&gt; 109

&lt;211&gt; 652

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (1)...(652)

&lt;223&gt; n = A,T,C or G

&lt;400&gt; 109

acaccccaaa	ctctcccttg	ggagcctcaa	tggcagtata	tgtggctcct	ggaggaactt	60
ggtagccctc	agtatacaac	ttaaagtgat	gaatcagtga	ctccatggaa	gtcttcatct	120
ctgctcgctt	aggtggagac	actttggcat	catcaacctt	gatctcccca	ggaggcatct	180
tgtttagaca	ctgtgcgata	attctcaggg	actggcgcat	ctcctccacc	cggcacagg	240

acctatcata	gcagtcctcc	cgagaaccaa	caggaacatc	aaactcaacc	tggtcgtaaa	300
catcataggg	ctgggtcttc	cgaggtccc	actggatgcc	tgagcccoga	agcatcactc	360
cactaaaaac	atagttaagt	gcttcttctg	ctgttacaac	cccaatgtca	attgtccgat	420
ttcgccagat	cctattgttg	gtcagcaact	cctccaactc	atcaagccga	agagagaagt	480
tcttagaaaa	ctgataaatg	tcatccataa	gccaaggggg	taggtcctgg	tgactcctc	540
ctggccggat	ataagcagca	tgcatctggg	ctncagacac	ttcgctcgta	gaactcaaac	600
atcttctncc	tttcttcaaa	cagccagaag	aaaggggtca	tgggcccaag	gt	652

&lt;210&gt; 110

&lt;211&gt; 96

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 110

acacattgag	tattccacag	atatacatgg	tttaatatgt	ggatatccatg	gggtatgatt	60
ctaccacagc	cttgtaagtg	ctccaaacct	taaagt			96

&lt;210&gt; 111

&lt;211&gt; 371

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 111

acatagcagc	ttcataacag	tttacttttt	taatataaag	atTTTTcaat	ttacacttgt	60
aggagtagaa	aaaactaata	tgctaagtct	gtaagctacg	cagcaaaaat	aatgatctta	120
atgaagccag	aattctgtga	aaatgtgcac	cacactgcat	atatagtagc	tgagtaaatg	180
taaacctatgt	gcttattaac	tcttctatat	aaaatattga	acccccaggt	ctcacacatt	240
gcctcctatg	tccacatcac	ttttctgaag	acagcctcat	gctttaagcc	aatatatatt	300
tgctatttga	aaaagttctc	atcctcatta	ctaaaaatgt	ttctgtaaag	gccttagaca	360
tttttttcag	t					371

&lt;210&gt; 112

&lt;211&gt; 406

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 112

caggtacagt	aatacacggc	tgtgtcctcg	gttttcaggc	tgctcatttg	cagaaacaac	60
gtgtcttctg	aatcatctct	tgagatgggtg	aatctgcctt	gcacgggtgc	agcgtagtct	120
gttgctccac	catcagttgt	gcttttaata	cggccaaacc	actccagccc	cttccctgga	180
gcctggcgga	cccagctcat	ccaggcgctca	ctgaaagtga	atccagaggc	tgacacaggag	240
agtgttaggg	acccccagg	ctttactaag	cctccccag	actccaccag	ctgcacctca	300
cactggacac	cattttaaatt	agcagcaagg	aaaatccagc	tcagcccaaa	ctccatgggtg	360
agtcctctgt	gttcagtcct	gatcactgaa	tgaaaacact	tgaggaa		406

&lt;210&gt; 113

&lt;211&gt; 492

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 113

accatcccca	gaagtgtctg	gtgccaggca	ctgatccagc	agctcttcca	caatggatga	60
caataaccga	agctcccat	tttcatcacg	ctggctgatc	tttgattgaa	tgaatctac	120
aaattcctgg	ctgctcatca	cattccagat	gccatcacag	gcaatgacca	tgaattcatg	180
gtcgtcagtg	agagtcagca	ccttgatgtc	aggaagggtc	gaaatcatct	gttcctcagg	240
tggcagggtc	ttgtttctct	tgtagaagtg	gtccccaatg	gctctggaga	ggttgaggcc	300
cccgttgact	cgcccatcca	tggtgacctt	gccaccagca	ttcttgatgc	gtgctagttc	360
tacttcatcc	tctggtttgt	gatcatagga	catgtctaaa	gctttgccag	cctcagatac	420



cacacagcga gagtctcctg cgttggctac aatcaactgc ttctctcgta tcagggccac 480  
caccgctgtt gt 492

<210> 114

<211> 234

<212> DNA

<213> Homo sapien

<400> 114

acctcagtgc aaaagttagt tgaactgggt cattcatctc tatggtaaca gtttcctcct 60  
ctttatcgac attacttgtc tgtgacaatt taatgtttcc atttccaagt tctccacttg 120  
cagaaaattt cactccgtct tttgcacagg aaattacaac agcatctcca atatggctga 180  
gatctcggca tatacgtgca aattcaccag aaggcatctt tactacacag ctgt 234

<210> 115

<211> 368

<212> DNA

<213> Homo sapien

<400> 115

cctggggtgg gatcagagga tctggcgtgg catcccgtag ccagtcatgc ctgcctgaga 60  
cgccccgcgg ttggtgcccc tctgtaaccc gatcaggttc ttgccctctt gcagctgggt 120  
atccgagaag ttccgaggat tctccttgga tttcttaggg aaccagttgg gatcccaga 180  
gaagagccca tcatctcggg ctactgccag cccaccaga ttcacagcg tccgctgcac 240  
acaggccatg ttctttcctt cccagaggtc cacagtttgg aagatgtcag tgggtgtaat 300  
gccatagcgc tcagctgctt gcaggaactg agagatctgc tccatctgct tgaaggccat 360  
ggtggagg 368

<210> 116

<211> 487

<212> DNA

<213> Homo sapien

<400> 116

ggatttttta ttgtgttttc cacatagata aaaaaataag gctttttgat gaaaagaatc 60  
cattacaaag tcaaaaatcc attacaatta taattgaatc agtaacaaaa tttagcttta 120  
aatgagtcaa gtattctgca tttgaaattt aatatcacia acattcaaga ttagtgaatt 180  
ttgtaagaa aaaaatacta gaagaaagga aaaggacacc ttttcaacag atagtaattt 240  
ataaaaattt ttttaaaagt gctttgggaa aacacacagt atcattactt aagaaaagtc 300  
atttaaggaa gacttaagtg cttcaagtgg agtgtattac agactaaaaa atgttttaaa 360  
atttgccaag aaatttaagt gttaaaaata ctcttctcct tattcagttt catgtttaag 420  
gaaacatttg acagacaagt aaaccaaacg caaaaaaaag ttcacctgca ttttaaaacta 480  
ataaatt 487

<210> 117

<211> 430

<212> DNA

<213> Homo sapien

<400> 117

gttttacttg ttgatttttg gatgcatgct gggggaggaa agcatattgt ttgtagtcac 60  
cctagagtgc taaggatat tattcccag taattctctc aagggtgggca tatgcaaac 120  
ataatctcta aattcttcaa tactaagaaa tacctttgtt ttacccttaa aatcaaatgc 180  
cattttggct ggatatagga ttctaggatt aaagcctttt tccagcagaa ctttgaagac 240  
attgctccat ttacttctag catccagtgt gtccagtgat aagtctgctg tcaacctgat 300  
tcttggtcct tggtaggtaa tttctcttct ctctctagaa gcccttatta ttttctctt 360  
atcactagaa ttccaaaatt tcaccaagat gtgtctagga gtcagtctct tttcatcaat 420  
tttactaggt 430

<210> 118  
 <211> 305  
 <212> DNA  
 <213> Homo sapien

<400> 118  
 cctgctagaa tcaactgccgc tgtgctttcg tggaaatgac agttccttgt tttttttggt 60  
 tctgtttttg ttttacatta gtcattggac cacagccatt caggaactac cccctgcccc 120  
 acaaagaaat gaacagttgt agggagaccc agcagcacct ttcctccaca caccttcatt 180  
 ttgaagttcg ggtttttctg ttaagttaat ctgtacattc tgtttgccat tgttacttgt 240  
 actatacatc tgtatatagt gtacggcaaa agagtattaa tccactatct ctagtgtctg 300  
 acttt 305

<210> 119  
 <211> 367  
 <212> DNA  
 <213> Homo sapien

<400> 119  
 cgggtacaaga catcaaagtg aagtaaagcc caagtgttct ttagcttttt ataatactgt 60  
 ctaaatagtg accatctcat gggcattggt ttcttctctg ctttgtctgt gttttgagtc 120  
 tgcttttctt tgtctttaaa acctgatttt taagttcttc tgaactgtag aaatagctat 180  
 ctgatcactt cagcgtaaag cagtgtgttt attaacatc cattaagcta aaactagagc 240  
 agtttgattt aaaagtggtc ctcttctctc ttttctactt tcagtagata tgagatagag 300  
 cataattatc tgttttatct tagttttata cataatttac catcagatag aactttatgg 360  
 ttctagt 367

<210> 120  
 <211> 401  
 <212> DNA  
 <213> Homo sapien

<400> 120  
 acaggtaaat aaaagatcac cttgaattaa actggatctc cttaagggca tagtatagtt 60  
 tcagtttcat tacctattac ataattagtt tcttacatac aaatattgac atatttggt 120  
 tgtgttctga agcctttgtg tctatgaagt ccacatcaat gcagctcata actggaagtc 180  
 actggggagt tctttgctgc tgctgggttt aacctgatca tgcattagag tctcctcagc 240  
 acctgttggt gctctgcaca cctctggggc atcgtcagtg tcaggatcca agccttcagg 300  
 gcagggaagt ttcagcaact cttcgcggag ctgagcagtg tgacgcttga gagctgctgc 360  
 atggtgagac atagtcctgc ctaccgctt atcactgctg t 401

<210> 121  
 <211> 176  
 <212> DNA  
 <213> Homo sapien

<400> 121  
 acagcccaga tgtgatattt ctacaggaag ttattccccc atattatagc tacctaaaga 60  
 agagatcaag taattatgag attattacag gtcattgaaga aggatatttc acagctataa 120  
 tgttgaagaa atcaagagtg aaattaaaaa gccaaagat tattcctttt ccaagt 176

<210> 122  
 <211> 443  
 <212> DNA  
 <213> Homo sapien

<220>

<221> misc\_feature  
 <222> (1)...(443)  
 <223> n = A,T,C or G

<400> 122  
 actgctgcca gttccccacg tggcccagcc ccaccacag gctctcctgg gccaggaat 60  
 gtccctgcagg agggaggagt cggtttccaa tgccagccgc cctaacaacc caggaaactca 120  
 gctcaactgg ttacagacct cgagttttca gccatgtta cttgaaggag aagcagttct 180  
 tgggctttac cacctgccac ctgggccaga gttctcttat ccttatccta agagtcttta 240  
 agactcaaag aagaaaaggt cttgtctgat gtataatctt aaaataaacc cacacttagc 300  
 cacctcaaatt cctttctgaa attatgtaag atgaaaactt aaatgcctta tagataccaa 360  
 gtatctcctc acaatattga attccatgaa accacttatc tttgcatgca atgaagcatc 420  
 cacaaaacca tttcaagctg aan 443

<210> 123  
 <211> 520  
 <212> DNA  
 <213> Homo sapien

<220>  
 <221> misc\_feature  
 <222> (1)...(520)  
 <223> n = A,T,C or G

<400> 123  
 actgtatatt ngaagattgc taagataatg gattttaagt gatctcacca caaaaaaaga 60  
 agtatataag gtattagata tgtaatttag cttgatttag ttattctaca aggtatccat 120  
 atatcaaaac atcatgttat ataccatgaa tatagacagt ttctgtcagt taaaagtaaa 180  
 taaaaatttt aaaaaattat caattcgtaa attttaccaa gttggggcaa aagcctttta 240  
 acagtccang aaatatttaa agctagtcaa cagcttctac agagatgaag aacattntgt 300  
 cctaagggtt ttctgtaggg atcaccocca tctctagact tctacctggt aaacacgcct 360  
 tccactgggt gatgaganta aggtgatgga ctgtcgatca actaggncca aggcctgggt 420  
 agctgatgag ccaaagagaa acttcagcct gtgaaataaa aacacttcag attagaangc 480  
 ctgattctca aagtcacctc agtaacttgc ccaaggatcc 520

<210> 124  
 <211> 406  
 <212> DNA  
 <213> Homo sapien

<400> 124  
 actaaaaatc aattggatga actaaatcca aaacatgaca ctgtaggcag cagttttaag 60  
 tcttattttt actgtttata tatttgaatg ctgtacaac agatgatctt catccctgaa 120  
 gttttcagct aaacttggtt tcctagaata gactgttaac tttcaaaatt tttattggtg 180  
 aaatggaaat actgtttttc cttgtgaatg aattttcata tttgtaagt ctaagtttat 240  
 aattcaggtt tgatcaaggt gtgaataact gaagaaaata acttgctggc tatataggaa 300  
 aatgctgtgg aaatgaactg tgtatatact tctggggagga acaaatttaa tcatttcttc 360  
 tgtaagcac taatcagtat aagtgaact cctggttctg tacctg 406

<210> 125  
 <211> 413  
 <212> DNA  
 <213> Homo sapien

<400> 125  
 gttttctttg aatgatttct ttttttcaact gtaagacact cctttaaata atgcctatct 60  
 ttaacttttt aagactattt ggaaaaatgc agtgtctcag ctgtccccag ggaaattaag 120  
 tggaattcaa ctaagatctg ttaataagat gtcagaataa ctaataattt tattaggaaa 180

aaatcatggt	ttaaatttca	aaatgacact	tatttgtcaa	gtaatatgat	cttgaaaaat	240
tttaaagaaa	aataatccta	cttataaact	acttttttat	aattgttttc	agaaaaaaag	300
tttacagtct	taaggaaaat	attcaggtct	atcatatggt	ttgacagatt	ttttaaaagt	360
tatttttgggt	aagggtcttct	tttagaaaaa	aattaatctc	aagggttttt	tgt	413

&lt;210&gt; 126

&lt;211&gt; 655

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 126

gtattctata	gtgtcaccta	aatagcttgg	cgtaatcatg	gtcatagctg	tttcctgtgt	60
gaaattgtta	tccgctcaca	attccacaca	acatacgagc	cggaagcata	aagtgtaaag	120
cctgggggtgc	ctaagtgtg	agctaactca	cattaattgc	gttgcgctca	ctgcccgtt	180
tccagtcggg	aaacctgtcg	tgccagctgc	attaatgaat	cgccaacgc	gcggggagag	240
gcggtttgcg	tattgggcgc	tcttccgctt	cctcgctcac	tgactcgctg	cgctcggtcg	300
ttcggctcgc	gcgagcggta	tcagctcact	caaaggcggg	aatacggtta	tccacagaat	360
caggggataa	cgcaggaag	aacatgtgag	caaaaggcca	gcaaaaggcc	aggaaccgta	420
aaaaggccgc	gttgcctggc	ttttccata	ggctccgccc	ccctgacgag	catcacaaaa	480
atcgacgctc	aagtcagagg	tggcgaaacc	cgacaggact	ataaagatac	caggcgtttc	540
cccctggaag	ctccctcgtg	cgctctcctg	ttccgaccct	gccgcttacc	ggatacctgt	600
ccgcctttct	cccttcggga	agcgtggcgc	tttctcatag	cttcacgctt	gtaag	655

&lt;210&gt; 127

&lt;211&gt; 442

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (1) ... (442)

&lt;223&gt; n = A,T,C or G

&lt;400&gt; 127

accttatggt	ccttgaaagg	aagactcaat	acttccagga	gtcaaagtta	atttgaatga	60
aaatggaaga	gaacaagttg	acaataattt	gaagcaattc	atgcttctag	ggctgaatga	120
cgtttagatc	agacacagag	tgactgagcc	aatcaacagg	catgtagtgt	gatctttccc	180
accacagtga	acagagggat	tctttgtcca	aggcaggctt	gcagctcggg	ccagcttgag	240
catttgatca	ggatttgatg	cttcaaagat	gaccactct	ctgtaaaact	attaccaaaag	300
caaaatgcaa	tgatctcttc	catttgtgga	acataccacc	aacacaaacc	acgcgtgggt	360
ttgcctcctg	ttcactccat	tttcaaggct	agagaaaggt	caagtccaaa	acaacagtta	420
aggntaaaac	gctaaacctc	aa				442

&lt;210&gt; 128

&lt;211&gt; 447

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 128

gtaaaatctg	atggtgggtta	aatgacgatg	tttaggtttt	gataaattta	gattttatac	60
acatgataga	gcattgtatct	gtatttttaa	aaataaagac	agagaactta	tgtttagaac	120
aagagaagcc	atttggtaga	aataaagaag	gagattgggg	aaggagatga	gaatgagtca	180
gagagatagc	atttaaaact	tgaaatcagg	cacaacaatt	agtatgtcat	gatataaaca	240
gtattgagat	aaaattttac	cacttctctt	ccctttaata	aattgtcaaa	ggataaaagt	300
tcctgtttga	aaatatattt	tactggtatt	gtgctttcct	catatcacag	attggtaaag	360
aatcatttta	agtccaagac	tcttatttta	catattctgc	aattaaagg	cctatgaggc	420
tacctgccga	ctgctgacat	gtagtgt				447

<210> 129  
 <211> 175  
 <212> DNA  
 <213> Homo sapien

<400> 129  
 ttcagacttt gttttagtc agccttggtt tggcttcaga ctttgtttgt cgtatttgag 60  
 gatataaata ttcataaata gtttcccaag tctggagcga ccacataggg agaaaatgta 120  
 aatgtctcaa tttttgttca caaaagtata ttttatcaaa ttgctgtaag ctgtg 175

<210> 130  
 <211> 406  
 <212> DNA  
 <213> Homo sapien

<400> 130  
 acatttacat tcaagttgat aacactggtg gtttcatttc aatacaaaatt atgctagaga 60  
 actgacattt cagacatggt catatatatg ctatttgaat tcctttatct tgatacagat 120  
 cttgattgtg aatctcttga tgatagatgt gcagctaatt tgtcccgaag ctcatgaaga 180  
 taattgtatt gcttgatggt ctgtattgcc ccggatcctc ttaggtctcg caggctgtct 240  
 atggcttgct ctggtgatat tgtgtcagac aggtatagta ggagacaagc agctacaaga 300  
 caagatctcc caagtcctcc atagcagtgt attaagggtt ttcggtaatt ttaaggcag 360  
 gttgtaagct cttccattat ttcacagcag ctggctatgt caggag 406

<210> 131  
 <211> 403  
 <212> DNA  
 <213> Homo sapien

<400> 131  
 accgcattac attatgcctg tgaaatgaaa aaccagtctc ttatccctct gctcttgga 60  
 gcccgtagcag accccacaat aaagaataag catggtgaga gctcactgga tattgcacgg 120  
 agattaaaat tttccagat tgaattaatg ctaaggaaag cattgtaatc cttgtgacca 180  
 caccgatgga gatacagaaa aagttaacga ctggattcta tcttcatttt agacttttgg 240  
 tctgtgggcc atttaacctg gatgccacca ttttatgggg ataattgatc ttaccatggt 300  
 taatgttttg gaagagcttt ttatttatag cattgtttac tcagtcaagt tcaccatggc 360  
 cgtaatcctt ctaagggaag cactaaagtt gttgtagtct cca 403

<210> 132  
 <211> 479  
 <212> DNA  
 <213> Homo sapien

<220>  
 <221> misc feature  
 <222> (1)...(479)  
 <223> n = A,T,C or G

<400> 132  
 cgaggtacag ggggacccc ttctcaacgg caccagcttt gcagacggca agggacaacc 60  
 ccagaatggc gttcgacca aacttagatt tattttctgt tccatccatc tcgatcatca 120  
 gtttgcattt cttctcttct tctgtgacgt tcagtttctt gctaaccagg gcaggcgcaa 180  
 tagttttatt gatgtgctca acagcctttg agacaccctt ccccatatag cgagtcttat 240  
 cattgtcccg gagctctagg gcctcataga taccagttga agcaccactg ggcacagcag 300  
 ctctgaagan accttttgag gtgaagagat caacctcaac agtgggattc ccgcgagagt 360  
 caaagatctc cctggcatgg atcttgagaa tagacatggt gaacttctag ccactgggtc 420  
 tcgtgcgcta ggagaggaag cggaggggtg tgcanaacac gaggtgaacg taaagcccg 479

<210> 133  
 <211> 301  
 <212> DNA  
 <213> Homo sapien

<400> 133  
 gtcttacagt gtgactcaga ctccctatct ggggatcggg taggttgctt caatctaact 60  
 atcaaaggac acgccaagtg tgtggaattt gtcaagagct ttaacctgcc tatgctgatg 120  
 ctggggaggcg gtgggttacac cattcgtaac gttgcccggt gctggacata tgagacagct 180  
 gtggccctgg atacggagat ccctaataag cttccataca atgactactt tgaatacttt 240  
 ggaccagatt tcaagctcca catcagtcct tccaatatga ctaaccagaa cacgaatgag 300  
 t 301

<210> 134  
 <211> 494  
 <212> DNA  
 <213> Homo sapien

<400> 134  
 actaagtgtg tacgtatttt tgccactttt tcctcagatg attaaagtaa gtcaacagct 60  
 tatttttagga aactgtaaaa gtaataggga aagagatttc actatttgct tcatcagtg 120  
 taggggggagc gtgactgcaa ctgtgttagc agaaattcac agagaatggg gatttaaggt 180  
 tagcagagaa acttggaag ttctgtgtta ggatcttgct ggcagaatta actttttgca 240  
 aaagttttat acacagatat ttgtattaaa tttggagcca tagtcagaag actcagatca 300  
 taattggctt atttttctat ttccgtaact attgtaattt ccacttttgt aataattttg 360  
 atttaaaata taaatttatt tatttatttt tttaatagtc aaaaatcttt gctgtttag 420  
 tctgcaacct ctaaaatgat tgtgttgctt ttaggattga tcagaagaaa cactccaaaa 480  
 attgagatga aatg 494

<210> 135  
 <211> 448  
 <212> DNA  
 <213> Homo sapien

<400> 135  
 actgaactcc catcacaaca tcctcttctt ctaataactg taacacaaca ctttcaataa 60  
 actttgcatt gggctctgcc atagctgctt tccggagact catgatgaat cttccgtgat 120  
 ggaaagctct tccactctgc acttgattgt tttctgacag agggtaagga atctgaacct 180  
 ctgatttgct ttctgatca tgaatcatgt aaccatttac aacctgggca tcaagacct 240  
 ccactgtatc tccaagacca aggtctttga gaacatgata accaccggc tgcaggaatt 300  
 ctccaactat tctgtcaggc tcttttaagt ctctctcaat gactgtcacc tttcttccat 360  
 ctctggaag cacagctgcc aaagcagagc caagcacgcc agctcccacg atgataactt 420  
 ctgggtcatt ctgagaagat gttgatgt 448

<210> 136  
 <211> 527  
 <212> DNA  
 <213> Homo sapien

<400> 136  
 accatgggtg cagcaatttc ttccataact tcgtggtaat ggtaattaaa agccatttca 60  
 atgtccaaac caacaaactc agtttagatgt ctatgggtat tagagtcttc cgctctgaat 120  
 actgggtccaa tagagaaaac cttctcaaaa tcagcacaaa tgcacatttg cttatatagc 180  
 tgtggggact gagccaggta tgcattattt ttaaaatatg acacagtaaa aacattggct 240  
 cctccttcac tggcagctga aataatttta ggagtttgga tttccacaaa acctttgtta 300  
 attaaagttt ctcggaagag atggcagatg ccagactgga gacggaagac tgctgacta 360  
 gttgatgtcc taagatcaat gactctgttg tctaactctg tatcctgggt aacagtagct 420  
 cttccttctt cttctccttc tgcctcaggc cgaacagcat catccagctg caggggcaga 480

cggggttcag ccaaactgat cacataaatc ttctgaacat gtaactc 527

<210> 137  
 <211> 275  
 <212> DNA  
 <213> Homo sapien

<220>  
 <221> misc\_feature  
 <222> (1)...(275)  
 <223> n = A,T,C or G

<400> 137  
 acgacgagtc gggccccctcc atcgccacc gcanntgctt ctaaacggac tcagcagatg 60  
 cgtagcattt gttgcatggg ttaattgaga atagaaattt gccctgggca aatgcacaca 120  
 cctcatgcta gcctcacgaa actggaataa gccttcgaaa agaaattgtc cttgaagctt 180  
 gtatctgata tcagcactgg attgtagaac ttgttgctga ttttgacctt gtattgaagt 240  
 taactgttcc ccttggtatt tgtttaatac cctgt 275

<210> 138  
 <211> 354  
 <212> DNA  
 <213> Homo sapien

<400> 138  
 caagctcaag gtgtttctgt caggaatgcc agagctgcgg ctgggcctca atgaccgcgt 60  
 gctcttcgag ctccactggc gcagcaagaa caaatcagta gagctggggg atgtaaaatt 120  
 ccaccagtgc gtggggtctc ctgcctttga caacgaccgc accatctcct tcatcccgcc 180  
 tgatggtgac tttgagctca tgtcataccg cctcagcacc caggtcaagc cactgatctg 240  
 gattgagctc gtcattgaga agttctccca cagccgcgtg gagatcatgg tcaaggccaa 300  
 ggggcagttt aagaaacagt cagtggccaa cgggtgtggag atatctgtgc ctgt 354

<210> 139  
 <211> 527  
 <212> DNA  
 <213> Homo sapien

<400> 139  
 acgaggaatg acctctaggg cctgggcaac agccctgtat ggccattggt ccacaccagt 60  
 catggccttg gatttttctg tcaaggcatg ggccacagcc atctcggagg cccaccccc 120  
 tggcaccagc tgagggtcca ggagaacatt gcgacacact tgcattggcat cctggagggt 180  
 gcgttctact tccgagagaa tctctttgct agccccccgg aggagaatgg tgcaggcctt 240  
 ggggtccttg cagtcagtga tgaaagtaaa gtattcatct ccaattttct tgatttccaa 300  
 caggcctgct cctgttccaa catcatcttc tctcagttcc tctggtcggc tgactatccg 360  
 ggccccacag gctctagcaa tgcgattatt gtctgtcttc cggactctgc ggatggctgt 420  
 gatattggcc cgcataaggt agtgctgagc taaatctgag atgccctttt cagtgatgac 480  
 cacatcgggc ttcagttgga taatgtcctc acagagctgc tggatgt 527

<210> 140  
 <211> 396  
 <212> DNA  
 <213> Homo sapien

<400> 140  
 acgccactgt ctottagata taattatccc caccctctgc tcatttggtt cccagattca 60  
 atacattgtc aaagcctctt ggtccttttt taacatctca cacttggtgc attctctcca 120  
 ttccataaaa cctcaacaac tgctcaaaagt cctgcttgac cccttggtgc cagtctttga 180  
 aatctttctt gcatatgact gcctcattac ctctctaaaa tctagttcac tcgcctactc 240

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aagaagacac aggggcctac tgtggtgtat tagataagtt cacatttctt ctctttacta 300
atctttttta cttcctttac caccactccc ttatataatt ccatcatcct aatagatctg 360
ttccctaca catccctgcc tctccacccc acatgt 396

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<210> 141
<211> 490
<212> DNA
<213> Homo sapien

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<220>
<221> misc_feature
<222> (1)...(490)
<223> n = A,T,C or G

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<400> 141
acaaccagct gtgctataag aaagaggag ggctgacca taactacacc aaggagaaga 60
tcaagatcgt agagggaatc tgcctcctgt ctggggatga tactgagtgg gatgacctca 120
agcaactgcg aagctcacgg gggggcctcc tccgggatca tgtatgcatg aagacagaca 180
cgggtgtccat ccaggccagc tctggctccc tggatgacac agagacggag cagctgttac 240
gggaagagca gtctgagtgt agcagcgtcc atactgcagc cactccagaa agacgaggct 300
ctctgccaga cacgggctgg aaacatgaac gcaagctctc ctcanagagc cagggtctaaa 360
tgccacatt ctcttctgtc ctgctgttcc ttctccttta tggacgtcta gtccttgtgc 420
tcgcttacac cgcaggcccc gcttctgtgt gcttgtcctc ctctcctcc caccataa 480
ctgttcttaa 490

```

```

<210> 142
<211> 511
<212> DNA
<213> Homo sapien

```

```

<400> 142
acatccagtc tgtatttctt acacaaaatt acatctaaat atttgacatg aggtcatttg 60
ctatcataag ccatcactag gaacttctag tctgtctcac tcgattgagg ctacaatgtt 120
gttaggtgct atgaccacaa tgaatacaac agacagcctc tcagctgtgc tgcaaagtat 180
tcataaccaa aagaccatat ttcaaattaa atcatagtag cgaatgacat accatttaca 240
tattacaatc tgagcctctg aaacaggggg aacatataat ggtatccaga acatctttac 300
atcaaaataa cctatcatat tacaaagttt tcaattccaa aaagtgtaac agagttaaag 360
gcaactggtaa ctttgtccac tgttagagat taaaacttcc aaagcaaatg aaagaaccat 420
tggtcacctt taacgtggg aaagtggga aaaagaaccc caggaggaca cccaaacctt 480
ctctgtgtcc tctgtggaac ctggttttt t 511

```

```

<210> 143
<211> 463
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(463)
<223> n = A,T,C or G

```

```

<400> 143
actgcagtga ctcatcagag tagaaggagt attcaataag tgggacttct gtgtcgttaa 60
attgggcata tgcataaaaa gtgccgtttg gagaccacca cagagcagag taggcactga 120
agacttcctc ttcataaaac cagtcagtta ttccattata tattatatct tctttcccg 180
tccatgtgat tctgtaaactt ggtaaatttg gttcaatttt aacataaatg tcattgttcc 240
aaacatatgc caatttatga cccactgggtg accatgtgac ccactgtgtg ttgtttggaa 300
tcctctcttc tgtaatcagc tgccttttat ttaaatcata aatgtcatat gaagctgtgt 360

```



aggaatgcct ccattgcttc acgtagtgtt attctaagag aataaactgc ccatcangag 420  
atattgaata atcattgata gaatgnccaa actcatcaaa tgt 463

<210> 144

<211> 297

<212> DNA

<213> Homo sapien

<400> 144

actcattaat attatthttgt tttgagaaag ccagaaatga ttctaagaaa taaacaataa 60  
taataaaaaga tgtaattaat atactgtatc ctttttaagc caaagcacac tttttacctc 120  
aagactgttc tgactttttac attcttaatt tcctttgtcc aaaataggac cccattttta 180  
atagagttca tttgaattga gttcataatc taaagtcact tttccccaca agatgttttc 240  
atttcagtat ataaactgct aagcggcaaa tgactaagtc agttataaag aatttgt 297

<210> 145

<211> 356

<212> DNA

<213> Homo sapien

<220>

<221> misc\_feature

<222> (1)...(356)

<223> n = A,T,C or G

<400> 145

actnctgcac ctcccttcagn aggaggncaa aggggaatgg cgacagctgc tcaatccttg 60  
tgatggncac ctgccccacc atgtcgctg ctttgcgctc cgggttgag gtcataatac 120  
actttgcccg tgcagaanag aagccttttg acattttctg ggntctgagc tgcaaggcca 180  
tcttctggga tcaccgcctg gaannnggtn cctggaagca tctcatcaaa gctggatctg 240  
gcctcggggg ggcncacaaan ggatttgggg gtgaagataa ttaacngctt ccggaatggc 300  
agcnggatct ggcgtcgtaa cacgtggaag aagctgccac gagngggagca nttgac 356

<210> 146

<211> 355

<212> DNA

<213> Homo sapien

<220>

<221> misc\_feature

<222> (1)...(355)

<223> n = A,T,C or G

<400> 146

acagttttgt tttctcgtaa ggggagcatc atagggttac tttataccag ttgtaacatt 60  
ttcattgttt ttggttgttc ttttttcttt ttttaatggc agctaaagat atacagatta 120  
ctgttaaatt gcagtccttt tttttttaa natattttct tgagttattt aaaacatggg 180  
aagcctggta ttttttaatc aaacaaaata tttatgaaan gggttttctc ttaattctgg 240  
attcatcatg gcttttcta accaattgta atatttacaa tattcaccaa aacttagaat 300  
tttgcaaatg ctggaattct gccagtgttt ctttgctaag cttgcatgc aaat 355

<210> 147

<211> 209

<212> DNA

<213> Homo sapien

<400> 147

attttttact ttatatatga aaatgtcatg aaatttataa gcaataatgt attgatactc 60

```

aaattttttaa aaattttttaa atttttaaaat atttaaatcaa cttctattat ttttcctctt 120
ctgggatgaa ttaagtggca aacttggcca ttctaataatt tactcactga tagccaaatt 180
ttatagcgtc tctatctaaa gaagacagt 209

```

<210> 148

<211> 445

<212> DNA

<213> Homo sapien

<400> 148

```

actcccagca aatcctctga atactccaca gactatgtta cccagtccca aggctattaa 60
ctcctgattg ccatcaagtg gataatcgta tttgagggaa tagacgctgg caactgaaaa 120
ggccactgca aatgcaacca ttgcgatgcc gaagcaatct cctacggtgt tttggaaagt 180
ctccacgtca ggtgtaatag ggggctgaaa tccaggattc atgtccccaa ccacagccac 240
tttaaacctg tttttaaagt cacagccgta ggatacacct gctgcaatca cggtcataat 300
gaattcgatt ggaatgggca ctggaagttt gtctttgaag cgctgattta tttctttaac 360
aatggataca accaaaagga caatcagagc tgtcaccagg tctgcaatat tagtcttctc 420
tatttgtgag aatacagagt atagt 445

```

<210> 149

<211> 585

<212> DNA

<213> Homo sapien

<220>

<221> misc\_feature

<222> (1)...(585)

<223> n = A,T,C or G

<400> 149

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actattaatg agaacgaaat acacattagc aaaatggagc catttcaatc tagtggtttg 60
ggcaagatgg ggaagagaag gggaaacatt ctagtttctg gattacatta ttatgccctt 120
cctgaaaagg tggttgtcat ttgcatttat ttaaagcagg taatatgcag gaatgtaact 180
gaggattatc ttcaggcaat cagcaagata tcctcctcat ggtcccttta gctctcaaaa 240
gcaatgaaat cctcctgttc tcatttttac tgctgtggtt gtgctgctga acaatactat 300
cttctcaaat tccatgccac aaattcagca ataacttttt ggattgaatt tagcaactac 360
tgtaattgga tgctgatgtg gacaaaatat attgatttcg atttcactcc cgaatgtgat 420
tgccaccagc tctttatatt gctgctgtgg tattttaaac cagaagcttc tttaaattat 480
gttgcaaaact gatctttgnt tttatgtttt ggtttggttt tatttctaag tgataagttt 540
gaaacacaca gctttaaatg atttttttat tgtgggattt tgggt 585

```

<210> 150

<211> 508

<212> DNA

<213> Homo sapien

<220>

<221> misc\_feature

<222> (1)...(508)

<223> n = A,T,C or G

<400> 150

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acaatgtctt agaaagtctt taagtcacat accatgaatt tttgcttcat tactgaccat 60
atatgacctt ggaggaaact tttttttttt ctttctactc atttctgttt ccacctacc 120
tgactcaccg tatttccagt cttctacccc tgcagttatc ctagtccagc aaagtcattt 180
ntttcaaaan anacatcatg tctgaaaata attactggta gtctaataatg agccanagta 240
aacagctcct catggtcaat gaacatgttc aggaagcgat caccttgatg cttgaaccca 300
accccanaca gnggacaatt ntactttgaa atatccngga atatttactg ggggatccaa 360

```

tttaaacttc	ttntttctnt	agcctttaaa	ttacacaact	ttgaactgac	acggatctnt	420
tacaaanaac	aatgcggcac	tgaaggaana	gatgattcct	ttactcaaac	ctgcaggaat	480
cagcctatta	acaggcaggg	gaaacggt				508

<210> 151  
 <211> 434  
 <212> DNA  
 <213> Homo sapien

<220>  
 <221> misc\_feature  
 <222> (1)...(434)  
 <223> n = A,T,C or G

<400> 151						
accatgaata	aaagtgcatt	tcaataccag	ttttaacaac	agcatatagg	gcagacataa	60
aagaagacca	cttccgaaac	tagtgcaaga	gattgagcat	taggcacaaa	gggagaaaaa	120
tgaaaagaat	gaactttttg	aaggaataag	cattaagact	agatgaccac	attattatag	180
agacaaagct	agcagcaaaa	ttttaatcct	tgatgatgta	gctttcaaaa	tttgcattct	240
ctcctatagt	ctaccctata	cgaacagctc	ttcctatttt	cctctttccg	actgtgaagt	300
tactaaatc	ctaactacta	ttccatata	tctgtgtgcc	aggcatttcc	catgcttgct	360
atctaactcc	cgggtaagca	aatcttgnag	taagaggcag	tacctgcctg	gcggccggtc	420
aagggcgaat	tctg					434

<210> 152  
 <211> 320  
 <212> DNA  
 <213> Homo sapien

<400> 152						
actttgcaat	catctttcct	tttttcacat	tggtaaaaat	aagtggcatc	cataggatca	60
tgatttttaa	tttgttgcct	ctgaagattt	cactccatca	agatctgcc	atcttcaata	120
ttctggctaa	atcttggtat	gtgggtttta	aacagtcact	ccgtttcaaa	gtctgtcttt	180
ccttatagaa	tgtggaaatt	atttctccat	accttgtgat	tttgacctga	gtgctaagag	240
aatcactctc	cttacctagt	tatctacaaa	tgttcattcc	agaaatgttt	agttactgaa	300
ttgaatgaag	acatctcagt					320

<210> 153  
 <211> 459  
 <212> DNA  
 <213> Homo sapien

<400> 153						
acctcatttt	tattagccat	tatcttcacg	ctggattcta	atattctttt	taatgggtgat	60
ctgttcaatg	acagaaactt	atagagagaa	aattccttct	caatttataa	acaaaaattt	120
taaaagcagc	atttttgatg	tggtaggaag	atatttatga	caaaagcagc	tactgcccta	180
aactggcaaa	aacaacaaaa	gaacaaattg	ttatttaacc	tttaataaac	gagtctctat	240
ttgctataaa	tctacaaata	ttttaaatat	atttcctcct	actgcaataa	aaattaagat	300
aactctctgt	ttaacagctt	ttgaagagtt	aattttataa	ggaaataaaa	aagattgact	360
tgctcctga	atgtccagtg	ataaactgaa	ccctaatttc	cctacctcaa	caacataaaa	420
atgatgtaaa	gtggatcaaa	gtatgtaaca	agttaatat			459

<210> 154  
 <211> 503  
 <212> DNA  
 <213> Homo sapien

<400> 154

acacagcctt	gttgccatgt	ctgttggtgg	ccacaatcgc	cttgtccttc	tgaattatga	60
tttctggaaa	ctcctgggcc	aggtgagtca	cttgaatggt	gcacttaatg	tgagagctgag	120
ctccttccat	gatcattccg	gtggggctga	tgtggaactt	gggtgtagag	aaggattccg	180
tcacgggtgac	cagttcactc	ttggtagatt	ctgaggtctg	catatggatc	ccagaaatga	240
tcctagcttg	acgtcggaag	gataaaacgc	ggtcctgttc	ctcaacgggg	aattccagta	300
tcacaaaatt	ctgggtctcg	gaattcttct	ctcttttcag	cttgaccatt	ttttcattta	360
gttcaagttt	ttcaattgtg	aagtgtattg	gggccttttc	ctctgggaca	gaacagttga	420
ccctcacgat	ccacacttgg	atggcctctt	tcttgtcacg	tgtcaccctg	ggactgggca	480
ctcctttcac	caacacctgg	tac				503

&lt;210&gt; 155

&lt;211&gt; 364

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 155

actaaatata	gaacacttaa	caaagtccaa	tcttttgctg	agtgaaaatt	taacaattta	60
ctgagagaaa	agtaaataata	agaattttaa	gttcctttca	tacttgatca	tactataagc	120
attgccatca	tttcaatgca	catatatttt	taaaaaacia	ttttctctct	caaactcata	180
ttaaataact	ggatttttaa	acattttccc	catccacaca	aaaaagatat	gtgggttcta	240
attattcttt	gctatttaat	aatgctacct	ttgaagattt	ctacataata	taaaccattcc	300
aattctgaag	caaagtattt	cagcattttt	caaagtctc	taatatatct	tttgtttgta	360
gcgt						364

&lt;210&gt; 156

&lt;211&gt; 452

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 156

acatatatgt	atattatacc	aatagctagt	aatttcaaaa	aaaacattga	cttgagtgtt	60
agataaccat	tctctaaatt	cagtttttga	tgtttcaaga	aacccaaaag	cctgtctttt	120
cacctacaga	ccctttgtgc	acgtggcaaa	tcacctctga	aaggcaaaaa	actaactgga	180
ttctcttcat	ttgttcaaaa	aagagaagaa	agctttaaag	atatgcctat	aaataaaaaga	240
aaatttaggtt	gctatattat	gattgtgcaa	taagtattaa	tttcattgaa	gtttgaccct	300
gttccatgta	ttagatgact	aagacattta	actcttaggg	atgttgaaag	cgcaccacaa	360
aacataagta	atcaataaag	taatgtttga	agacttttag	tatatactgc	ttattcaggt	420
aattaattat	tttgtaaata	ctaatagcatt	at			452

&lt;210&gt; 157

&lt;211&gt; 224

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 157

acatgaacag	caggctgttg	cattgtaact	tgtggctgtg	cattaagatg	ttgctgagga	60
ttgcgaactc	ctgcagcata	tttatactgt	ggaacgggtg	ggacagcagg	agtagctgca	120
gcggtgcag	ctgcaggacg	tggaaccatt	gtctgtgttg	atgtgttagc	aacacgctgt	180
gttgacatga	ctcgtggaac	ctgtgaagaa	gctggtctca	tagt		224

&lt;210&gt; 158

&lt;211&gt; 623

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 158

acacatttca	ttatgctgcc	ttttctctta	tgattaaaac	tttagccctc	attcgagggt	60
tccaatggtt	acttttagtg	gaggagtcc	ctagctttta	aaaaaccact	tttctcttaa	120

```

gattccatta tttattgaaa gaagtccttc tagaaatggt aaggaggatt ttaaataaac 180
acattcaatt aaaaaaaaaa tcacgtattg aacatctacc aagcatctgg actcttcgga 240
acctagtaaa atgaaaaaat ccagttttaa caacagtaac ttcattctgc gggatatacag 300
agacaagcac gtttcttctt ttggtctaatt ttattctaaa cgaagaagct gggaactgac 360
aaaacaggac aggttggttt taatccagtc taaaaataaa caagacaatg cctgagttag 420
ccctctatat agatttaggc ttatgctgac ctggttgtaa aatctgtatt taactaaaag 480
ttaataaaaa tacatatgtt cattttaaaa taattactga ttttgcttgg ctatcccacc 540
ccttaccccc aaactcatat atttttagga caagatttct ctgcataacc acaacctgtc 600
tcctccccc cacccccac ata 623

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```

<210> 159
<211> 422
<212> DNA
<213> Homo sapien

```

```

<400> 159
aggtaccatc ttcttcagaa ctgcatctaa gaggtgtgct tggctgggaa tcatacagct 60
gtgggcaaca actgcatcag ccccaaggct tcctccaga ccaaaagggtg attcatggcc 120
cctggttaat atcacccctag gttctccct gtccagttt taacataata ttcatagaa 180
atactagtgc cataaaaaat caacatttca aatataaaaa ttattttata caaatgtaat 240
tcataatcat tcttttaaaa tacagcattg ttatatatgt ttgaaacatt attaaaaata 300
atatttccta gagaaaaaat tttgcttcac aaaattataa aacagaagca tataaaacta 360
attcatgatt ggtgcttctt cagtgtgtct ctcattctct cttagtgtag acagcatgaa 420
gt 422

```

```

<210> 160
<211> 393
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(393)
<223> n = A,T,C or G

```

```

<400> 160
agctcactct tttatctgtg tggctgattt cattactgtt tgtgatttgg agctactcac 60
tggatggtga cctcttttca ctttctctac tccatgtctg ggcatagcc agctttggac 120
tccttgagcc cctctctaatt ttaaatttga tattattaat tatccaggta attgtcttcc 180
gtgtgggtgc ctcttccccc actccagtat ccactttcag caaaacgtct tgcttcaagt 240
cccagataga agagtctttg acttttcttc agaggcttat tttagctaga atgtttaaag 300
ctacagatgc ctatctgctc atcttccag ctggattagg tgttgcttag atttgctagt 360
tgctttaagt attacacagt ttttgnattt atg 393

```

```

<210> 161
<211> 223
<212> DNA
<213> Homo sapien

```

```

<400> 161
accacttaat tactggcact gagtatcact gaatttctta gttttctagt ggggaaacat 60
tattgagaag ccctccctta ttttaagtaa gttgattaaa tcttatgtga gttgccagtt 120
gtaatttttc aaaggaaaaa ttttgatggg gtggagggaat gaattgccag ataattttc 180
tggaattccg agagaattcc aaagagggtt ttttttttt tag 223

```

```

<210> 162
<211> 487
<212> DNA

```

&lt;213&gt; Homo sapien

&lt;400&gt; 162

acaagtctac	attcccacta	acagtgttta	aacgttcctg	cctctgcatt	ctcgtcagca	60
tttgttactg	tcttttggtg	actgtcattc	taacgggggt	aagacaatct	ctcattgtgg	120
ttttgattct	ctttagaacg	aatattttctc	ctcattcctc	tactcttaat	aatggatttt	180
ctgaaaaaca	tctattaatt	ttatgcacta	ttcaattcaa	acaacttttt	aaaagttgcc	240
aaatctgtca	caaaatatta	aacaacaaga	aaaatatcta	aaggtaaact	tgagaggggt	300
gtaaaacaaa	agactctgag	agcgcaacta	gctgtaaaac	aatcattcct	attcctaaat	360
tgagtgtttt	tggttacatg	ttctaagtgc	cttacaataa	accaggcaat	gtgctttatc	420
tgagaaaagg	gagccctaac	ttcaaagttt	gagttcctcc	aactttttta	atagttaaat	480
ttcaagt						487

&lt;210&gt; 163

&lt;211&gt; 500

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 163

acactggatg	cagccatgca	tgatgggttt	ttctttattt	ttcagtgatt	tcctctgaag	60
cagctgcact	gatacatttg	ggagtgggtg	gcttgacttt	gtccataagg	ggcgtggcca	120
cttcacatga	tggggggcct	ttaagagcac	aaagaagttt	aatatggaca	acaacaggaa	180
aaagcaagaa	gaaaacaagt	agggaaaaac	agctaaccctg	gagagaaaaga	atttctttaa	240
cctttatgtt	cttcattaaa	aatcttatct	tggtactgatt	tgagggattt	ttagaaacat	300
ggccttattt	tatataagca	ttaccttccc	aggaatcttt	gttgatatatt	aatttttgat	360
aaccatttga	ttaactttta	aattaagtat	atgtgtgtat	atatacatat	gtatgtttat	420
atacacacat	gtatctgtat	agttttatat	atacatatat	acacatagac	atacagagaa	480
ccactacttt	gtaatatgtt					500

&lt;210&gt; 164

&lt;211&gt; 547

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 164

actgtaatgg	gtttggccaa	atatcatctt	tgatgacctc	tcctaactca	tcagcacctg	60
catcagaatg	gtcagtaaac	caggtaaaga	agctctctgg	ttcctcatgc	tgccctcttc	120
tgctggcttt	attctgcgtt	tgactcgaa	gtttcgtcaa	atcctttcca	gatttccatt	180
tgattttcgg	ggacttcgaa	gatggatcac	cactctcatt	cagatgaaat	tctttggaga	240
gaactttatt	ttcaaagtaa	ggattttcat	caaaataaaa	atctattctg	taacctgatt	300
taatatcttc	aaattctgtc	acttcaactc	tggtcaaaata	atgcagtgcc	tcttcatctt	360
cctccccaag	cagtgcagac	acttgtggat	ggttgacaaa	tggtgttacc	caaaaatttg	420
ggattttggc	gatcaattct	gacctcttct	gaaaaaatgg	ttggcggagt	ttgttatatt	480
tctgttctac	tttcaaaatc	tcctcactgg	cttgttcatt	aagtctgtct	atttcatttt	540
gtacctg						547

&lt;210&gt; 165

&lt;211&gt; 400

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 165

acaaaactta	caaagaagtc	aaaagtctta	acactcccat	tctccaggaa	ctcttgtctg	60
tgctcatctg	taggaggag	gaatcctgg	tcctcaggt	ccttgtcatg	ttagtctttt	120
gatagcttca	atccactcgg	ctcgtcggc	cttgctgctg	gcctgaatgt	aatagtgtgt	180
gtcatcctta	gtaatcactt	tgaagagggt	tcctggaca	ttccctttaa	ccccagtggg	240
aacgccatta	tcttccagag	cagacacgag	tgaaccacga	agagaaaacc	caccactggg	300
cctgttctct	tctttggaag	ggtcatagta	atgcaggaaa	gctggatcct	tccttagaac	360

aaagcgacgc accttccagt ttttcctctt gtgccttgc

400

<210> 166

<211> 274

<212> DNA

<213> Homo sapien

<220>

<221> misc\_feature

<222> (1)...(274)

<223> n = A,T,C or G

<400> 166

ggtaccttca	tataataaag	ttacaaaaa	taataaaata	ttaaaaaaa	gagccagctg	60
gcactgccaa	ccaattccta	tagtagcctt	agaaatccta	atcctgtaga	atttcctctt	120
gtagtcaata	agcaccaccn	tcttcaggag	tatttcagtg	tattgttatc	tacaccaagc	180
aagcctgggtg	atgcagctac	ctgagttctc	ttggttatgg	gtgaatgta	tcttcattca	240
taacttcccn	gctttcatgt	aggtggggat	agag			274

<210> 167

<211> 478

<212> DNA

<213> Homo sapien

<220>

<221> misc\_feature

<222> (1)...(478)

<223> n = A,T,C or G

<400> 167

ctttttaaaa	tccaatatat	tctgccaa	atatgccttg	atagttagcc	ctcagcccat	60
aggtgttttt	tgttttttta	cagaattata	tatgtctggg	ggtgaaaaaa	cccttgcat	120
ccaaagggtcc	atactgggta	cttggtttca	ttgccaccac	ttagtggatg	ttcagtttag	180
aaccattttg	tctgctccct	ctggaagcct	tgcgagagc	ttactttgta	attgttgag	240
aataactgct	gaatttttag	ctgctttgag	ttgattcgca	ccactgcacc	acaactcaat	300
atgaaaacta	tttaacttat	ttattatctt	gngaaaagna	tacaatgaaa	attttgntca	360
tactgnattt	atcaagtatg	atgaaaagca	ataganatat	attcttttat	tatggtaaaa	420
tatgantgnc	attattaatc	ggccaaatgg	ggagnggatg	ntcttttcca	gnaatata	478

<210> 168

<211> 213

<212> DNA

<213> Homo sapien

<400> 168

acaaatgtaa	cagtaatgat	aaattctctt	ttccaaggga	aagagaaacg	ctgcagaatg	60
gacattaaac	aaggcattat	gccctacaag	caagacataa	aatgtctaag	ggaaacttca	120
gcataaaaaat	gttgaacaca	taatgtgaga	taatttgaat	aaataacaac	tgacattctt	180
tttttaaaaa	aaaagtataa	aaaatagatg	tgt			213

<210> 169

<211> 341

<212> DNA

<213> Homo sapien

<400> 169

actggctgcg	aggcgccagt	cgatcaatgt	atgacaggag	ctgagacttg	gccacaccag	60
gatcccccac	cagacagatg	ttgatgttgc	cccggatttt	catgcctcga	ggagactggt	120

```

ccacaccccc gactagcagg agcagcagtg ccttcttcac atcttcatgc ccgtatatatt 180
ctggggcgat tgaagctgcc agcttttcgt agaaatcctc ctctgcaatt tgcctcagct 240
cctccctggt gagctctcca gcccagact catcatcctc actcttggtc atcttcacaa 300
tccgatgggc ttccaggtag gtttctgaga gtaaaccttg t 341

```

```

<210> 170
<211> 543
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(543)
<223> n = A,T,C or G

```

```

<400> 170
accaatgata atgcttccat ttttttagt tttaaaccac caaaccaata tttttccttt 60
aaattttaat cttataatat agaaatctta tgtaaataa attttgcat gtttcaaata 120
aagagaactg aagtagaaaa tagaaatgcc agtaaacaac ataatgttta atttacaact 180
tacattaggg gtttggggga atgctaatta tatattgaga atatacatta gaactcttca 240
aaatgggctc ttctaataag gtcactactg aacaaaattg ttccctcttc tgtaaataag 300
aatagggtta aatgactagt caaatgaatt attttcttct tgtaaataa attaatctt 360
actttctttt aatgaccaac cttagggtaaa acaaaaatat tgtaatccta gaaattatcc 420
tccagctttc tcacctgaaa atctattgaa gtgatccctg gtcacccata taatgggatg 480
aggggaagtt ccagcagatt tcaggctgnt cttaaagggt ttggtggnca ttttctcaat 540
agt 543

```

```

<210> 171
<211> 280
<212> DNA
<213> Homo sapien

```

```

<400> 171
acatactaaa aatattttaa atagagaata ttccctcacag aggacttttt tctttaatta 60
ctactaaaaa aataattaca aagtccaaac aggcagagag atttagcaca ctgatcacac 120
gattctccat catcctccac gcttgctctg aagagggttt aaaaagtcca gtttctcggt 180
gatttcgctg ctccatttag ccaagggttg cctggccact gattggcaca agtgggtaat 240
gcgcttgat aggtcatgtt tgtgtcttgg aaatttgggt 280

```

```

<210> 172
<211> 463
<212> DNA
<213> Homo sapien

```

```

<400> 172
caggacttat ttacctatt aataagttcg gtctctgctt gcaatctttc cattgctcca 60
gcataccagg gttggcaaga ataacttact ggtttgaggc cacatgggca aggcttgact 120
gcatcacttg gaaaaaatcc aacctctcca gatgctaaat ttctgacctg ccaaacaga 180
ctgtgtgcat ctcttttcag aagttcaacg gtatccccgg cctggagctg taaagggggt 240
ccttcagca gagctggggg tgggtgtcca gaatagttcc taatgacctg catctttggt 300
aaacctggat ccacctgttt aggagttctt cgcagtcctt tgggtccgtt ctctggtagt 360
ttgagtgtcc cttgttctga aagaaatgta aaaattggca ttgtcagtggt aaagttattt 420
tgttttggtta gcaaccttag ctttctctgc agagtggttaa aac 463

```

```

<210> 173
<211> 165
<212> DNA
<213> Homo sapien

```



<400> 173  
 acccaaagaa ctggtggcct caggccacaa aaaggaaacc caaaagggaa agagaaagtg 60  
 agaagaaact gaagatggac tctattatgt gaagtagtaa tgttcagaaa ctgattatgt 120  
 ggatcagaaa ccattgaaac tgcttcaaga attgtatctt taagt 165

<210> 174  
 <211> 532  
 <212> DNA  
 <213> Homo sapien

<400> 174  
 actccatctc ttgactgaa taggtcattg atcctatcaa gggataacaa tgtttttgcc 60  
 actggatggt gatgttccta tccaaatcca cagcaagctg gtgttgcaat tttccagatt 120  
 catgcagatc cactgacttc agtgtgttga tactggcttt gaagtattcc atccactggc 180  
 ggatcgttga atctccatt aggtatatga gttttctctc caggcattcc ttcattttga 240  
 ctgtagccaa actacaggag acaggattcc atgtgtttct ccagacatgc ccactgggga 300  
 ttgtgatgt cattccaaac ttgcattttct ctttcattgc aactgtttct ttgttgcat 360  
 tggagacact aattgtattg aatttttcca taatctctac acccacattt gacctttcaa 420  
 agaggctctt ttcttgtttg ctaagataag aaactttctt gttcttagaa tacatgtgag 480  
 tgagtgcagc acagggcatg tgttgaggcc tcacacagta gaagccttct tg 532

<210> 175  
 <211> 374  
 <212> DNA  
 <213> Homo sapien

<400> 175  
 taatcacctg actgagctcc aattaactga ggagaaacgg ggtggaggag agggctggtt 60  
 gctattcaga cttgataatg agattgatct gtcccatgga gagtgaaggt tcagttccac 120  
 ttctgcctcc ttctttccat gctgtcctca tgctctttat cctcacttcc tcagtccctt 180  
 caacactcaa aatctgattt tttttctctc tcacacgtat caggggcagt ttctgaagtt 240  
 gctgaggttg aattttcttc acaaacctct ataaaacatc agcagagaaac atataaatac 300  
 attttgatta gcatacattg caaaatttct cccaacaatg caggggatga aagcaggtgg 360  
 tcccactga gagt 374

<210> 176  
 <211> 428  
 <212> DNA  
 <213> Homo sapien

<400> 176  
 actgcaactg ccagaacttg gtattgtagc tgctgcccgc tgactagcag ctggactgat 60  
 tttgaataaa aatgaaagca ttaaagggtt tccctacaaa acatttttct ttaaataact 120  
 tttgaaatgg ctataagcag ttgactttca cccttgagga gcatcacact gtgtgaggtt 180  
 cagtgatgtg tgacctccc cagccctccc tgctctttta agttatctgt gtgcgtgcgc 240  
 ttctctcaa tcttctttgc acgtcattt ctttttctct gacctatgag aaaggaaaac 300  
 ttactgatga taatttttaa atagtgtaat ttattcattt atagcatgtc aggataaatt 360  
 aaaagaacat ttgtctggaa atgctgccgg gagcctattg tgtaaatgta ggtattttgt 420  
 aaaataac 428

<210> 177  
 <211> 318  
 <212> DNA  
 <213> Homo sapien

<400> 177  
 acctgaacga agtcgcgggc aagcatggcg tgggccgtat tgacatcgtg gagaaccgct 60

tcattggaat	gaagtcccg	ggtatctacg	agaccccagc	aggcaccatc	ctttaccatg	120
ctcattttaga	catcgaggcc	ttcaccatgg	accgggaagt	gcacaaaatc	aaacaaggcc	180
tgggcttgaa	atttgcctgag	ctggtgtata	ccggtttctg	gcacagccct	gagtgtgaat	240
ttgtccgcca	ctacatcgcc	aagtcccagg	agcgagtggg	agggaaagtg	caggtgtccg	300
tcctcagggg	ccaggtgt					318

&lt;210&gt; 178

&lt;211&gt; 431

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 178

acttgaggct	tttttgtttt	aattgagaaa	agacttttga	attttttttt	aggatgagcc	60
tctcctagac	ttgacctaga	atattacata	ttcctccagt	aagtaatact	gaagagcaaa	120
agagaggcag	gattgggggtc	acagccgctt	cttcagcatg	gaccaagtgg	gccttgggga	180
ttgcagcggt	ctcgaagtgg	ctgtaggact	cgaattttaca	gaaagccaca	gaggtgcaac	240
ttgaggctct	gctagcaagc	caccagttag	gctattgggt	aaccaccttt	ctatacagga	300
gattggaatc	tactttgtca	tttatccacc	acagtgacaa	aggaaaagtg	gtgccgttat	360
gcaatccatt	taactcataa	acataattact	ctgagtaact	ggccagccat	tcacgcggatc	420
cttcattggg	t					431

&lt;210&gt; 179

&lt;211&gt; 323

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 179

actgccact	tttacacaag	ctgcagcaga	actcagttct	actgcagggtg	agagtattgc	60
accatcatta	acataataag	gacctcagaa	tccaaccttg	ccaaagaatt	caactcctag	120
gctcagatta	atggaagtgc	tgggcacatg	ccacctctg	ccattgtcac	agttcagctg	180
tgtggccccc	gacacagctc	cagttccacc	catgacatct	ggctgaggag	gcttatggga	240
gcggcttctc	atgcacagtt	actgtccctc	tctggagggt	cctttaatgg	ggactgtgca	300
aagcagtgac	actaactgcc	agt				323

&lt;210&gt; 180

&lt;211&gt; 409

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 180

actgtgttcc	tttgcattgt	tcttctttta	agaatttagc	tccttctgct	gtttctttta	60
atgcttcaag	taagccttca	tctgctttta	gtcttctatc	cttacttgag	ggataagttc	120
aatacctttc	ttggcttcca	caccagaggc	cagggcagcc	gtggtgggtg	gtctgagctc	180
agagctactc	tgaggggtca	catttgcttt	ggcgggtgtg	gcctttcctt	tcttgtcatt	240
tttggaagtg	tcaactgggca	cgtcggctat	gtcactagtt	tcaatgcccc	tagctctcat	300
ttggtctgct	ctcttttctg	taattgagag	aaatttcttt	ggatctgata	aagcatccac	360
gatatctcca	aatccatcag	gcacatatgt	tttaagaaca	atattgcaa		409

&lt;210&gt; 181

&lt;211&gt; 460

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 181

acaaagattg	gtagctttta	tattttttta	aaaatgctat	actaagagaa	aaaacaaaag	60
accacaacaa	tattccaaat	tataggttga	gagaatgtaa	ctatgaagaa	agtatttctaa	120
ccaactaaaa	aaaatattga	aaccactttt	gattgaagca	aaatgaataa	tgctagattt	180
aaaaacagtg	tgaaatcaca	ctttgggtctg	taaacatatt	tagctttgct	tttcattcag	240

```
<210> 182
<211> 232
<212> DNA
<213> Homo sapien
```

```
<220>  
<221> misc_feature  
<222> (1)...(232)  
<223> n = A,T,C or G
```

```

<400> 182
actgacagat taatggcttg cctagagctg tgcaagaaac agcctgccag nctgtcattg      60
nnagggacca gggcaaaacc aagagctgtt cttccagaa gagccctgca aacacattgg      120
ttcgtgtctt cctttacttc ttctggctcg ataccatgaa tgccagtcac cagtaaaatct      180
taatacactt ttgcttttatt ctccacatgcc attcaccaga ttatttgatg gt              232

```

```
<210> 183
<211> 383
<212> DNA
<213> Homo sapien
```

<400> 183						
atgtttattta	aaagatgaaa	tttcatggtt	caaatgtatt	tttctcccat	aaaaatattt	60
tctcttccat	ttaaatatat	acctaatctt	tgagaaatct	tgacaaaatg	gcattttatt	120
aaagaaaatc	taattttacaa	agctttgtta	atgttgagaa	aaacattctat	agatcataaa	180
caaaaatttc	aatatgcaat	attcaaattt	acaagaaaat	aagcacaaac	ttttagacag	240
tgcagtattt	gctgcactcc	tttaattcct	tatccagagc	ccaaaaaatg	taggcaaacc	300
ctaaaaatgt	agcagaagca	tttccgcaca	ctggtgtcca	gaatctagtt	tgtgcagaaa	360
tctttccact	aqattttataq	aqt				383

```
<210> 184
<211> 444
<212> DNA
<213> Homo sapien
```

<400> 184						
acagacacaa	acatataaat	atatgtatgc	acatatattgt	catacatttt	caataaatga	60
tatctttaat	attgtttaa	gacctttttt	ctcttgtgaa	ttttgacata	aagtatattt	120
tataaaaata	gagagttggt	gacttacgat	gtatttttga	taatacaatt	tgtatctctt	180
ctgctctcat	tgtgttgatg	tttgcctaaa	atgtcttctt	ccacttgcca	ctttcaggct	240
gatttcacta	ctagatctca	agtgactctt	gaagagaggc	aagttggatc	ttggtatata	300
aaattttata	taatccctct	attcaatgta	tgtgtattga	ttggcaagtc	tattttttaa	360
atattttatt	tctgaagaca	aagattactg	ttattttatt	gtttaatgat	tcttgtagg	420
ctgtttctca	tcttatcttc	cttt				444

```
<210> 185
<211> 289
<212> DNA
<213> Homo sapien
```

```
<400> 185
acttgtgaca ggcagacgtg attgcagcca cgaacacgat gaactcactg aagtccacct    60
gggcattctcc attggcgctcc aggtccttga gcaattttatc cacggcatcc ctgtcttttc    120
```

cactctgcag	gaagcctggt	agctccttct	ccatcagcac	cttgagctcc	cccttgggtca	180
gggtctgcgt	gctgccctcg	ctgcccgaat	atcgggaaaa	gacgtctatg	atcatgcccc	240
tgactgtctc	tagttccgtc	atggtgctag	attcagaccc	accttcctc		289

&lt;210&gt; 186

&lt;211&gt; 407

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 186

acagacaaaa	tgctcaggat	gccatgattg	ccctagagca	tggatcacct	tcccagcaat	60
cggttttctg	caggatgcac	aatggccctt	gggcaactgt	gcaatgccaa	ggtcctgcaa	120
ttcctgctcc	agacccccaa	gcattgagtc	cagggaggcc	ttgtgatcct	gcttgtctgg	180
taagtgtctc	ttgccagcat	ctgctctcac	tgcaaccttg	gcctgcatct	cagtcagggt	240
agccatgagc	tcatccaact	gagcagctgc	tgacgtttta	gaaggtgggt	gtgattcctt	300
tggtctcttg	gcttcactgt	agacattgag	ctcctggata	ttggtagtat	acacgagctg	360
cgccggcaag	ggacttgtgt	tatcctgaat	agaaaggatc	tccgaag		407

&lt;210&gt; 187

&lt;211&gt; 441

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 187

actgcaagac	ccatcttccc	tccagttaat	acactccag	gatgggctgc	agagggggag	60
actctgagag	aagctggagg	cccacaaaag	tccactgacc	ctctttctgt	cccagaaatg	120
aataaaggac	ccagttgtgc	tttccttcca	aaatcctcaa	caaagtgtgt	tgtgtctcaa	180
gaaaatgtgg	gaataaaaaa	atcatgtccc	aggatcatct	tgtgtgtgtg	cggggggagg	240
ggatgggagg	aaaaggcatg	tattaataga	tactgctgct	ataaaatgac	ataaatcata	300
gcccttgatc	tgtttctgta	aacaatgcca	gcttcttcag	gttattggca	actaccctta	360
atatacctag	cccagatcct	ttcataaagt	caagtgtctat	atttccaaaa	taatcctatg	420
aatcatgaa	ggttgtgaag	g				441

&lt;210&gt; 188

&lt;211&gt; 323

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (1)...(323)

&lt;223&gt; n = A,T,C or G

&lt;400&gt; 188

acttagaaaa	cagtcocctgt	ccatcagcca	gaaaagggtga	ccatcacccc	taaagtaatt	60
tccaaacttt	agttcagtg	gaaagatatg	ctggtagtgc	atattcagng	ntgattttca	120
gtgctagtaa	ccacttttaa	tgccagaaat	atgtaacaat	gataatgtaa	cgtaaaagt	180
gttactaaag	attatagcct	taactttttt	atgnaaaaga	taaaatccat	tcctcctccc	240
agttagcaag	catggcttgc	atttctcaaa	aatgagaact	tccatggcag	ccaagaaaac	300
gtcttctcag	aggaactttc	gtt				323

&lt;210&gt; 189

&lt;211&gt; 225

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 189

caggtagctcc	ctgatctttt	cctcagtggc	ttcaggattc	agacccccaa	cgaagatttt	60
-------------	------------	------------	------------	------------	------------	----

```

cttcaccggg tcctttctca tagccatggc ctttttaggg tcaatgacac ggccatccag 120
cctgtgctcc ttctgggtcta ggaccttctc cacactggct gcactcttga acaggataaa 180
cccaaaccct cttgaccgtc cagtgttggg atccattttt attgt 225

```

```

<210> 190
<211> 501
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(501)
<223> n = A,T,C or G

```

```

<400> 190
acagctgaag ttngataaca aagaaatata tataagacaa aaatagacaa nagttaacaa 60
taaaaacaca actatctgtt gacataacat atggaaactt tttgtcagaa agctacatct 120
tcttaatctg attgtccaaa tcattaaaat atggatgatt cattgccatt ttgccagaaa 180
ttcgtttggc tggatcatac attaacattt tcnagagcaa atccaagcca ttttcatcca 240
agtttttgac atgggatgct aggcctcctg gnttccattt gggaaatgta ttcttatagn 300
cctgtaaaga ttccacttct ggccacactt cattattggg agtgcccaaa gctctgaaaa 360
tcctgaagag ttgatcaatt tctgaatccc catggaaaag tggtttctta gttgctagtt 420
cagcaaatat ggtgcctata ctccaaatgt caactggagt tgagtaacga gctgacccca 480
gcaatacttc tggagatctg t 501

```

```

<210> 191
<211> 436
<212> DNA
<213> Homo sapien

```

```

<400> 191
acagtgcatt gtgctgtcac ttggaaagcc tttcaatgtt gtcttcagat tgttgtgatg 60
aatatgaaac atgcagaccc tcctttataa agaaaaagac cttaaaactt gaatatgaga 120
taattttaca ttttaaaagt ttatttgatt ttcatattat tcactttcaa agccctttca 180
aatagaaaag gtatgaactt ttgggggat aatttatgta tcgtaaactt attagaacaa 240
aatattcctg atgtataatg agttgtttta tttatacaac tttttcaatg gtagtttgca 300
ctattcttta ttatgctaca ggtttattta ttatgaaaca aaggaaatag tattttatgt 360
attttaccat gcataaggta actctttgac acagatttat tggttcttga tacacctaaa 420
ataaaaaaaa atgtgt 436

```

```

<210> 192
<211> 319
<212> DNA
<213> Homo sapien

```

```

<400> 192
ccagcgacag actttgcaaa catgcagatg gttctcacat gtcttccttg tctcattttc 60
agggcacgtg tcctaggttc tttcgattac gtctctcaag gcaagggttc cagatctctc 120
tgtatcctta cgcttccctt ttggatgcac ctttaatttta aaatacctct ttttctcatt 180
aattagatca cttcaagtta aatacaaaac atggcaagat ggatttaaatt ttagagggat 240
ataagtatac ataagagaag accaatctct acttttataa atgcagttaa ttaacaataa 300
agtaaaatat agtgaaggt 319

```

```

<210> 193
<211> 586
<212> DNA
<213> Homo sapien

```

<400> 193  
acaagaggcc atttgtcttg cctttttctg acatgtgcat actataaaat cacaggtagc 60  
caacatttag tatcagtaaa aaacaactac gtttgttcac ctgtttggca tagggagaaa 120  
acaatgtatc tcatagcatt aaatgataca gccttaacac atatgatgct catatttgca 180  
aagtccccaa atgttgagaa gttctagtga aaagtcatac tattgtgcaa agatgaaaat 240  
ttggggccaa tgtctgtatt caaaataacc aaaatatatt ttaaagcaaa atatatcctg 300  
atactactat agattctagg aattgtccta aaagagtaaa gtgttgtttc ctttctgaac 360  
atgaataaca tcaaaggaag aaccagttc ttaagactta agtaggaaat ttatagaaat 420  
ttgatttata ccagtagtaa taacattcat aaggaaaaac tattaggtaa caattttctc 480  
caagaagagg atcagattac ttaaaattgt tggagaattc tggttgtttg cgcaataatc 540  
atagtgattt acattgcttt tcttctttca gagcaataag aaagtt 586

<210> 194  
<211> 214  
<212> DNA  
<213> Homo sapien

<400> 194  
acatttttat aactggaatg tttatgtgta gtgaagctct gagaggactt tgcattagat 60  
ctcagcagca taatcagaag gttgtccttt gtctcagcaa tttttaagct aatagtagca 120  
gaaattgcag tggaaataga ctgcttggcc acaacattca gaaaatcatt tatcttttta 180  
ttgcagttct tgtcaccaaa caatacattt tagt 214

<210> 195  
<211> 325  
<212> DNA  
<213> Homo sapien

<400> 195  
actgtacata tttgcaatca cattgtgcat agattcctaa tggtagatat gatttctttt 60  
gtcaggctac aacaatgaac tgcagattcc ttgtttgtaa tgtaaatgat tgaatacatt 120  
ttgttaatat gtttttattc ctatgttttg ctattaaaaa ttttataaca tttccaagac 180  
aaaaattcca agttttatgct ttgaagaatt tatgtaatta aaatttcaact aaactaatct 240  
ttttagttta ggaattattt gggttttgac actggaagtt gcgccaaata agcatcagaa 300  
ataggagatg cttaacattg ctata 325

<210> 196  
<211> 382  
<212> DNA  
<213> Homo sapien

<400> 196  
actccttccc agttttttct ttatactgag ccttcaggga cagtaagcat tctacagctt 60  
catttatttt agccttaggg gatttttcag ctttttagctt acgaaccacc tccccttggtg 120  
cagcaacttc atcatacaga gatttacttt ccagaatact tgctgaggaa ttagaagaaa 180  
tattctgtcc tatttcagca ggagggtttc caggtttata ttctggcca gttttctcct 240  
tatattcagc ttccaagac aaaagctggt ttacagctgc atctacatct tcctttgggtg 300  
ctttcttggc ttttaattca cgaaccacat ctcttgaac agccactcta ttgtaaagga 360  
ccaaggaatc ctcagatgta gt 382

<210> 197  
<211> 648  
<212> DNA  
<213> Homo sapien

<220>  
<221> misc\_feature  
<222> (1)...(648)

<223> n = A,T,C or G

<400> 197

acatccacat	gttcctccaa	atgacgtttg	gggtcctgct	tgccaacatt	ctttattgcc	60
agctgttcag	gtgtcatctt	atcttcttct	tctacagcct	tattgtaatt	cttggctaatt	120
tccaacatct	cttttaccac	tgattcattg	tgttttacaat	gttcactgta	gtcctgaagt	180
gtcaaacctt	ccatccaact	cttcttatgc	aaathtagca	acatcttctg	ttccagttca	240
tttttccgat	agttaatagt	aatggagtaa	taatgtctgt	ttagtccatg	aattaatgcc	300
tggtatagatg	gcttgtttta	gtgaccacga	ttcgaagttg	tttgtcttgg	ttcatgtcct	360
aagaccatca	tatttagcatt	gatcaatctg	aaggcatcaa	taacaacctt	tcctttttaca	420
ctctgaatgg	gatccacaac	cactgccaca	gctctctccg	acaaggcttc	aaagctctgc	480
tgagtgttgat	tatccacacc	agaaagccaa	caaccaaaagc	cagggtgact	gtgataccaa	540
ccaacaacca	tctccggcct	tctgtctgc	ttcaacatat	ccaacatttt	aacttggaac	600
actggtatcaa	ctgccttcac	actgacacct	ggtinctgatg	nggcatag		648

<210> 198

<211> 546

<212> DNA

<213> Homo sapien

<220>

<221> misc\_feature

<222> (1)...(546)

<223> n = A,T,C or G

<400> 198

acaatacagc	accactactg	agaagggtct	gaggttttgc	aatccaaggt	tctgacttaa	60
agcaaaaata	cacggcatag	attgcaacag	caaagaagtg	tccaattaaa	actagagggt	120
taggagacaa	tacagaaagc	agcccaacag	gacccgcaac	acattcgcca	ccaagtttga	180
aataaagaaa	acaggctttt	cttagttgat	gcagggaatc	atctgtggca	gaaaataatt	240
cataaagagc	ctgagcaagg	atattcacga	caaaggaatg	agatgttttt	cttgcccagt	300
aaaatgattt	tttggcctcg	aaaatagctg	catcatcata	aaggtcaggg	atacccttta	360
gcagttttct	ccatagtttt	atatctttta	aagcaacagt	cattcctcca	ccagtaagtg	420
gatgcctcat	atttatatgcg	tctcccaaaa	gaagaacacc	tcgtttcttc	actgatgaag	480
gaggaaggaa	gcttgctgca	tggacctcag	atgagaattg	cagtggttct	aagaatggtc	540
ntttca						546

<210> 199

<211> 275

<212> DNA

<213> Homo sapien

<400> 199

actatgtgta	actttggcaa	caggttgcag	tcagccaggg	tgagctcggt	gccatccaaa	60
aacttcctct	gagagacacc	ttcatcttca	gcactggttt	catccacttc	ttctgggagg	120
ggggatgta	agtaattgtc	taaaaccttc	agggttttca	ggagtccctt	ctccagattg	180
tcattgagtg	ctgggtttga	attcttgatg	taggcagaaa	atttggcaaa	tatgtccagc	240
ccagctgtgt	tggactcagg	gttcagagct	gccag			275

<210> 200

<211> 423

<212> DNA

<213> Homo sapien

<220>

<221> misc\_feature

<222> (1)...(423)

<223> n = A,T,C or G

```

<400> 200
cctgagaaat tctnaaaagt acgatgataa ggttgcaaaa atgaagaagc tcatcatact      60
aaaactagga aacatacnga tccataacan gacatgcnaa gcaaagttcc caaagtcaca      120
gacaagaaga gaattctcaa ttgctgaaaa tacataatta tgggtgcatg atntaaccag      180
tgactctttc aacataaaacc ttgcaggcca gaaggaaatt gcgtgctata gttgaggtgc      240
caagcgaaaa atagcttcta tgtaagaata acataaccag caaaactgtg ctacaaaaat      300
gaagaaaaag caaagacctc taaagataac caaacgtgga aaaattatat caacactaca      360
tgtgccatac aaaaaatgct gagaagagtc ctccatttaa aactatatga tgctaaaaaa      420
caa                                                423

```

```

<210> 201
<211> 560
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(560)
<223> n = A,T,C or G

```

```

<400> 201
acaatcgagt attttagaaa ttacatgaaa catgaaacag tttttgcaat tttttttaa      60
ctgggcatct ggtttctaaa aatttatttg aaacaatcta gaattttctt ggtgcaaagt      120
gtatcatgtg gaatatcctc atatttttac catattttta gaactttaag acgattaatt      180
gtaataaatt tatttgattg gtgcagttct aatccctaaa tcataatctt aaaatcagga      240
atgtgtggag aacagagcca tgcatatca ctttgctctt accattcctt ttgatcagcc      300
tcaattcagc ctcatgtgt agtatgtttt ttctttctat gaaaaacaac agaaagcatt      360
tcattttatt tgcctatgtt caaatatgtt taataatgac caaagtgcatt tctgagtttt      420
ttcaaggaat gtaatactgg agctttaaga acatacttag tttctcatgt gaaaacttan      480
gctttgtctg angttttcct tcctctattg nctaattggt aggtggtttt aggaattatg      540
ttttataact tttcaatata

```

```

<210> 202
<211> 366
<212> DNA
<213> Homo sapien

```

```

<400> 202
acgagcccca cagagcagga agccgatgtg actgcatcat atattttaaca atgacaagat      60
gttccggcgt ttatttctgc gttgggtttt ccttgccett atgggctgaa gtgttctcta      120
gaatccagca ggtcacactg ggggcttcag gtgacgattt agctgtggct ccctcctcct      180
gtcctcccc caccacctc ccttctggga aacaagaaga gtaaacagga aacctacttt      240
ttatgtgcta tgcaaaatag acatctttta catagtcttg ttactatggt aacactttgc      300
tttctgaatt ggaaggga aaatgttag cgacagcatt ttaaggttct cagacctcca      360
gtgagt

```

```

<210> 203
<211> 409
<212> DNA
<213> Homo sapien

```

```

<400> 203
cgaggtactg aagaacccca tcatgtgaga gatcgctcaa agtcattaac acaaagcagt      60
gaaaatcatc cagcaaaagca gtgctattat gagtgtgggc tatggaaaga cagcttttcc      120
tacctgata aagaaaaaaa aatgaggaaa ttatttcatc cccttgtagc atctgtgact      180
ttttggattt aataatcttg ctgtttttcc tctttatgac aaagaatata attgggagga      240
tgaagtgtct taaaaattgt agagaccagc tcactggaat gtttttccat ccctgtattc      300

```



```

atggccttgac tttgtgactg ctctacactg catgtctgac attgcagagt gagctatgtt 360
gaggtaaaact gggttggttgc attatatttgc aatcagcctg gtctctccc 409

```

```

<210> 204
<211> 440
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(440)
<223> n = A,T,C or G

```

```

<400> 204
acacacatcc tgatctagct atgtttatgt gtgttggggg gatggatgga caagagggtat 60
agttcaaatg agatcatttt tgtgaaatgg ctttgtaaac tgtaacatgc cctataaata 120
tgagatttagc ttaataactg gccctgactc tccagtgtgg ctttgtgtgt ttgtctaaac 180
acttagttaa tatctgtcag tgggtccattg cacaagggaac tgacacaatg gtatcctgtg 240
cctctgttgtg tgttgttgtt gttttttttg cagttctaaa agcttagtta attgccttca 300
ttagcttaat atataaccacg tgaaaagcat agaaaagcag aactcaaaac tcanagaata 360
aaggacagaa cataactaac tactgatgtg caccttagtt acctgatgca gggaattgaa 420
gcatataagc ttcacttagt 440

```

```

<210> 205
<211> 474
<212> DNA
<213> Homo sapien

```

```

<400> 205
acttgtecca tgctaggtaa caggaaaata atagtgattg ataagacata gtccctgtcc 60
tcaaagagtt aacagtctag caaggcagga actttgagaa aagaccaatg tgttcaaagg 120
aaaactcaca acctgggtct cccttctcag atggcacatt caagaaactg ttgcttatgc 180
ccctgggagc cagagcctta cttaagtctt accaagtcaa atatctatca gcctcagatg 240
atttgagcct ggtaaagtct tagcaataga tttgtgtgct catgttccca tgaaaaccta 300
ataagagaga gccctttcaa ctccaggcata cgggggggtt aaggataaca tgtttagtga 360
ccatgtggac attcagcaca ggtgagcttc tcaagtgaga gccatgtgtc cccaaaagaa 420
aggagggttt atccataaga ctttgccttc cttttcaaca ctgtgtgtgg aagt 474

```

```

<210> 206
<211> 344
<212> DNA
<213> Homo sapien

```

```

<400> 206
accgtccttc ttggggcaga tgtctgagat aaactgttcc acgccccag ccaaaccaca 60
gcagttcaac gcatagtgga tggctttcag cgtttccgc tggggctcat ccttggtttt 120
cagcttggtg taggtgtcct tgtaaaactc ctggacttcc ttaatcacct catccttggt 180
ggaatatccc cagatggccg cagctatttc aatggcgaat atcaccaaga ggaagccgaa 240
gaacagtccc agcatgcact gggactcctg cacagccccg cagcagccca ggaagcccac 300
cagcatcatg agggcgccgg ctccgatcag aatatagact cctg 344

```

```

<210> 207
<211> 441
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature

```

&lt;222&gt; (1)...(441)

&lt;223&gt; n = A,T,C or G

&lt;400&gt; 207

acctcaatttt	ttcccccaat	ttctggctac	tactaaaagc	cagaaagaac	agaacagtgg	60
cctcaggaga	tctgagtttg	aatccttgct	ctctaggatg	caggtggctt	gaagcagaat	120
gccacacctg	caagttgatt	agaactgcct	ttcttcccag	gcttgacata	ggtattaagt	180
caaaattaca	tgaaccacag	tggtaaaaaa	gcctctgaaa	gctgtaacac	cctcagtaat	240
aacaaaagg	atttttat	cacagctaaa	gggaaaatag	gtggagaagt	taaaaaataa	300
tgtctgatcc	tgctcctaag	ttccaaacta	tagccaacac	tctgatgctg	ctctttttct	360
tgtaggacca	accgtcccag	tttgccctggg	actttctcat	ttttacagag	tcccaaattcc	420
tangaaactg	gagcaactgg	t				441

&lt;210&gt; 208

&lt;211&gt; 365

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 208

ctgggtgccag	tgccagtgtc	tgagccagtg	ccagagccgg	aacctgagcc	agaacctgag	60
cctgtttaag	aagaaaaact	ttcgccctgag	cctattttgg	ttgatactgc	ctctccaagc	120
ccaatggaaa	catctggatg	tgcccctgca	gaagaagacc	tgtgtcaggc	tttctctgat	180
gtaattcttg	cagtaaatga	tgtggatgca	gaagatggag	ctgatccaaa	cctttgtagt	240
gaatatgtga	aagatatatta	tgcttatctg	agacaacttg	aggaagagca	agcagtcaga	300
ccaaaatacc	tactgggtcg	ggaagtcact	ggaacatga	gagccatcct	aattgactgg	360
ctagt						365

&lt;210&gt; 209

&lt;211&gt; 191

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 209

cgagggtacag	aatataaagg	agactgttga	attcatacca	tataaaactt	gtagggtttt	60
taaaccatagc	aatcaaggct	acaaaaacaa	acctgtgttg	tttttgtata	gattgtaggt	120
ttatttttgg	atttcatata	catgactgaa	ctgtgtgcaa	ggcaatagtt	agccttgatt	180
ttagccacaga	g					191

&lt;210&gt; 210

&lt;211&gt; 373

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 210

acttaattgt	atatttcatt	taaatagtcc	ttctcagggg	tttaataatt	tagaatcaat	60
agttcccttc	aaaacataat	aaaatatatta	cactttataa	aatattaacc	cgattaacaa	120
tacagccgtg	ttgtttataa	gagtgttaact	gaagtccctgc	aaatcatgct	gttgacacaa	180
gcctgtgagg	ttagcgaagt	gataccttagc	aaaatgtaaa	tgaagatctt	cagacagtgg	240
tgttttataaa	atagctcatt	aatgacttag	gattgaatcg	ctccaacccat	tcgcatcatc	300
agatataata	atagtgcaga	atcagacagg	aaagatcctg	gctaaacccat	ttgcattttt	360
ttccagaagt	acc					373

&lt;210&gt; 211

&lt;211&gt; 336

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 211

actgtaatct ttcttcatca aaatatgcaa aacagcatca tggattgtta agaaaaatat 60  
tgagcttttc acttcacccat caaaaaattc ataccgggta agcttctcaa tgaagtcata 120  
atcagttcca acgatatata catctacctt gatcctgata aattcttgca aaatcgattt 180  
aaggccctc actgaagaaa catcaagaaa ggacactgct gaaaagtcga gaatgaggct 240  
gtggaggctg attttgggga cctcaatgtt gagaggaaga tcatcattcc agtcaatgtg 300  
gaaaggcagg tctgtggtat tgattgctgg tccagt 336

&lt;210&gt; 212

&lt;211&gt; 434

&lt;212&gt; DNA

&lt;213&gt; Homo sapien

&lt;400&gt; 212

accaccagca attttaagga aatcttcacc tgttgctttg taaacctcaa tataccgggt 60  
ccccatgtga tgtttgtgcc tctgtagtgc taggtctcgg tgctctcac ttacaaacct 120  
aaccagagct tctccgttcc ttcgaccctg agcattcaga caaagtgtg caccctccct 180  
ggcaatattg agtcctttga agaactcttg aatatcttga tctgaagact gccatggtaa 240  
acctcgtgcc ctgactacgg tgttatcacc aataagttcc atcttgctgc aagtccact 300  
ttcaaaactg taattcactc tctctggatc tgaaaacctg tgattataag gctctgaaat 360  
cattgctaaa attatattcc ccatatcttc aacttgagag gctccatata gagagactga 420  
actactcttc tcaa 434

&lt;210&gt; 213

&lt;211&gt; 515

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 213

actacacgac acgtactctt gaatacaagt ttctgatacc actgcactgt ctgagaattt 60  
ccaaaacttt aatgaactaa ctgacagctt catgaaactg tccaccaaga tcaagcagag 120  
aaaataatta atttcatggg actaaatgaa ctaatgagga taatattttc ataatttttt 180  
atttgaaatt ttgctgattc tttaaatgtc ttgtttccca gatttcagga aacttttttt 240  
cttttaagct atccacagct tacagcaatt tgataaaata tactttttgtg aacaaaaatt 300  
gagacattta cattttctcc ctatgtggtc gtcacagact tgggaaacta ttcatagaata 360  
tttatattgt atggtaatat agttattgca caagttcaat aaaaatctgc tctttgtatg 420  
acagaatata ttgaaaaca ttggttatat taccaagact ttgactagaa tgtcgtattt 480  
gaggatataa' acccataggt aataaaccca cagg 515

&lt;210&gt; 214

&lt;211&gt; 353

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 214

acaagactca agtaaataga aaggcagctt tcaatcacaa atcagttttt cagattttac 60  
tgtggaagca tatttaatgc acacatttga atgttacaca taaataattt taacgatgga 120  
gtccaagttc tggattttac attagatctg catatataag acatttgtgg tcaaatttca 180  
agatttgtaa agccagtttc aagctgctta tattttgagt acctgcccgg gcggcgctaa 240  
gggcgaattc tgcagatata catcacactg ggcggccgct cgagcatgca tctagagggc 300  
ccaattcgcc ctatagttag tctgattaca attcactggc cgtcgtttta caa 353

&lt;210&gt; 215

&lt;211&gt; 699

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

<221> misc\_feature

<222> (1)...(699)

<223> n=A,T,C or G

<400> 215

```
acacttgaaa ccaaatttct aaaacttggt tttcttaaaa aatagttggt gtaacattaa 60
accataacct aatcagtggt ttcactatgc ttccacacta gccagtcctc tcacacttct 120
tctgggtttca agtctcaagg cctgacagac agaagggtt ggagattttt tttctttaca 180
attcagtcctt cagcaacttg agagctttct tcatgtgtgc aagcaacaga gctgtatctg 240
caggttcgta agcatagaga cgatttgaat atcttccagt gatatcggct ctaactgtca 300
gagatgggtc aacaaacata atcctgggga catactggcc atcaggagaa aggtgtttgt 360
cagttgtttc ataaaccaga ttgaggagga caaactgctc tgccaatttc tggatttctt 420
tattttcagc aaacactttc tttaaagctt gactgtgtgg gcaactcatc aagtgatgaa 480
taatcatcaa ggggtttgtg cttgtcttgg atttatatag agcttcttca tatgtctgag 540
tccagatgag ttggtcacc ccaactctgg agagggtctg gggcagtttg ggtcgagagt 600
cctttgtgtc ctttttggtt ccaggtttga ctgtggtatc tctggccaga gtgtaggaga 660
nggccacaag gagcaagaat gctgacactg gaattttct 699
```

<210> 216

<211> 691

<212> DNA

<213> Homo sapiens

<220>

<221> misc\_feature

<222> (1)...(691)

<223> n=A,T,C or G

<400> 216

```
ncgagggtaca ggtttcacta ttacaaatat atgatgttaa actaacaac tcattgacctt 60
caaagatgtc ttcggtccac gcacacacat ttgtaatttg tgtccatttg ctatttcctt 120
tcttctataa tcttcaaatt atatagttat gcattgagtt ccctatgcat ctcacccatc 180
tcctttatct cagccttctc atactttgcc attctcttct ttctggaaat aaccagcaca 240
acaattccag caacaactgc tatcaccaca accacaataa cagcaataac accagctttt 300
agaccctgca ttgagaattc aggtgctttt tcatcaacat aataaattaa agtttgacca 360
ggatccagat ccagttgttc cccatttact gtcagggtcca ttttcttaga atgaaacaag 420
gattcacctt taacatcttt ttcaaaataa taagccacat cagctatgtc cacatcatc 480
tgagtttttt gagaagaatt ttgaaccaga tcaatagtga taacattatt ctcatacaaa 540
atactcgtga taaatttttg atccagttga taacgcgttg tgatctcctt ctgaagtgc 600
gtccgcaaac ttttactatc ataagggttt tctcttgctt tgnnggttag ttcaatggat 660
gatccagtag ggtctcactc gctcagagca a 691
```

<210> 217

<211> 497

<212> DNA

<213> Homo sapiens

<400> 217

```
ctgtgctcct ggatgggttt accacaagtc caattgctat ggttacttca ggaagctgag 60
gaactggtct gatgccgagc tcgagtgatc gtcttacgga aacggagccc acctggcatc 120
tatcctgagt ttaaaggaag ccagcaccat agcagagtac ataagtggct atcagagaag 180
ccagccgata tggattggcc tgcacgacct acagaagagg cagcagtggt agtggtattg 240
tggggccatg tatctgtaca gatcctgggtc tggcaagtcc atgggtggga acaagcactg 300
tgctgagatg agctccaata acaacttttt aacttgagc agcaacgaat gcaacaagcg 360
ccaacacttc ctgtgcaagt accgaccata gagcaagaat caagattctg ctaactcctg 420
cacagccccg toctcttcct ttctgctagc ctggctaaat ctgctcatta tttcagaggg 480
gaaacctagc aaactaa 497
```

<210> 218  
<211> 603  
<212> DNA  
<213> Homo sapiens

<400> 218  
acaaaggcga aagagtggat ggcaaccgtc aaattgtagg atatgcaata ggaactcaac 60  
aagctacccc agggcccgca tacagtgggtc gagagataat atacccaat gcatccctgc 120  
tgatccagaa cgtcaccag aatgacacag gattctacac cctacacgtc ataaagtcag 180  
atcttgtgaa tgaagaagca actggccagt tccgggtata cccggagctg cccaagccct 240  
ccatctccag caacaactcc aaaccctgg aggacaagga tgctgtggcc ttcacctgtg 300  
aacctgagac tcaggacgca acctacctgt ggtgggtaaa caatcagagc ctcccggtca 360  
gtcccaggct gcagctgtcc aatggcaaca ggaccctcac tctattcaat gtcacaagaa 420  
atgacacagc aagctacaaa tgtgaaaccc agaaccagc gagtgccagg cgcagtgatt 480  
cagtcacact gaatgtcctc tatggccgg atgccccac catttccct ctaaacacat 540  
cttacagatc aggggaaaat ctgaacctct cctgccacgc agcctctaac ccacctgcac 600  
agt 603

<210> 219  
<211> 409  
<212> DNA  
<213> Homo sapiens

<400> 219  
ctgagagacc aggagaagtt ccagatgcag agactgtgat gctcttgact atggaattat 60  
tgcgccagct agccaagtta gagacaaaac aggcgtaggt cccgttatta tttggcgtga 120  
ttttggcgat aaagagaact tgtgtgtgtt gctgcggtat cccattgata cgccaagaat 180  
actgcgggga tgggttagag gccgagtggc aggagaggtt gaggttcgct cccgaaaggt 240  
aagacgagtc tgggggggaa atgatggggg tgtccggccc atagaggaca tccagggtga 300  
ctgggtcact gcggtttgca ctcactgagt tctggattcc acatacatag gctcttgctg 360  
catttcttgt gacattgaat agagtgaggg tcctgttgcc attggacag 409

<210> 220  
<211> 635  
<212> DNA  
<213> Homo sapiens

<220>  
<221> misc\_feature  
<222> (1)...(635)  
<223> n=A,T,C or G

<400> 220  
acagtgatag ctccccctgg gcaatacaat acaagaacag tgggttttgt caaattggaa 60  
caaggaaaca gaaccacaga aataaataca ttggttaaca tcagattagt tcaggttact 120  
ttttgtaaa agttaaagta gaggggactt ctgtattatg ctaactcaag tagactggaa 180  
tctcctgtgt tctttttttt ttaaatttgt ttttaatttt ttttaattgga tctatcttct 240  
tccttaacat ttcagttgga gtatgtagca tttagcacca ctggctcaat gcgctcacct 300  
aggtgagagn gngaccaaact cttaaagcat tagngctatt atcagttacc accatttggg 360  
gcttttatcc ttcattgggtt atgatgttct cctgatgaca catttctntg agttttgtaa 420  
ttccagccaa agagagacca ttcactattt gatggctggc tgcatgcana catttaaagc 480  
ttttanagaa tacactacac cagggagtat gactactagt atgactatta ggagggtaat 540  
accaagagtt ggactacgca ccttaggcaa gatncaaacc anctaaaata gaataaagaa 600  
tgagtcagat gagtgtagcc attttaacca agcag 635

<210> 221  
<211> 484  
<212> DNA

<213> Homo sapiens

<400> 221

```
actccctggt ttgagaaact ttcttgaaga acaccatagc atgctggttg tagttggtgc 60
tcaccactcg gacgaggtaa ctctgttaatc cagggttaact cttaatgttg ccagcgtga 120
actcgccggg ctggcaacct ggaacaaaag tcctgatcca gtagtcacac ttctttttcc 180
taaacaggac ggaggtgaca ttgtagctct tgtcttcttt cagctcatag atggtggcat 240
acatcttttg cgggtctttg tcttctctga gaattgcatt ccctgccagg cctaccacat 300
accacttccc ctggaattgg ttgtcctgga agttctgctg cagagggacc ttgctcagag 360
gtggggctgg gatcagggtc gaggtggagt cctgggcctg ggcatgcaga gcccccaaca 420
gggctaggcc cagccacagg agacctaggg gcatgatttc agggccgagg aagcaggcgc 480
tgtg                                         484
```

<210> 222

<211> 566

<212> DNA

<213> Homo sapiens

<220>

<221> misc\_feature

<222> (1)...(566)

<223> n=A,T,C or G

<400> 222

```
acattaaagt gtgatacttg gttttgaaaa cattcnaaca gtctctgttg aaatctgaga 60
gaaattggcg gagagctgcc gtggtgcatt cctcctgtag tgcttcaagc taatgcttca 120
tcctctctaa taacttttga tagacagggg ctagtgcac agacctctgg gaagccctgg 180
aaaacgctga tgcttggttg aagatctcaa ggcgagagtc tgcaagttca tccccctctt 240
cctgaggtct gttggctgga ggctgcagaa cattgggtgat gacatggacc acgccatttg 300
tggccatgat gtcaggctcg gcaacaggct ccttggtgac actcaccaca ttgtttttca 360
agctgacttc cagcttgta ccttgagag actttagccg caccagggcc ccgatgcctc 420
cgctaaccag gatttcatca ccaatgtggt atttcaggat gttggcaagt tccttggcat 480
ctcccaagag tctgctccgt tctcttggtg gcagggtcgc gaaggcttca tttgtgggag 540
caaagactgt gtagacttcc tttccc                                         566
```

<210> 223

<211> 478

<212> DNA

<213> Homo sapiens

<400> 223

```
caggtaactta tttcaacaat tcttagagat gctagctagt gttgaagcta aaaatagctt 60
tatttatgct gaattgtgat ttttttatgc caaatttttt ttagttctaa tcattgatga 120
tagcttggaataaaataatt atgccatggc atttgacagt tcattattcc tataagaatt 180
aaattgagtt tagagagaat ggtggtggtg agctgattat taacagttac tgaaatcaaa 240
tatttatattg ttacattatt ccatttgat tttagggttc cttttacatt ctttttata 300
gcattctgac attacatatt ttttaagact atggaaataa ttttaagatt taagctctgg 360
tggtatgatta tctgctaagt aagtctgaaa atgtaatat ttgataatac tgtaatatata 420
ctgtcacaca aatgcttttc taatgtttta accttgagta ttgcagttgc tgctttgt 478
```

<210> 224

<211> 323

<212> DNA

<213> Homo sapiens

<400> 224

```
acgggcaccg gcttccccta cagatgggtca cccacctgca agtggatggg gatctgcaac 60
ttcaatcaat caacttcata ggaggccagc cctccggcc ccagggaccc ccgatgatgc 120
```

```

caccttgccc taccatggaa ggacccccaa ccttcaaccc gcctgtgcca tatttcggga 180
ggctgcaagg agggctcaca gctcgaagaa ccatcatcat caagggctat gtgcctccca 240
caggcaagag ctttgctatc aacttcaagg tgggctcttc aggggacata gctctgcaca 300
ttaatccccg catgggcaac ggt                                     323

```

```

<210> 225
<211> 147
<212> DNA
<213> Homo sapiens

```

```

<220>
<221> misc_feature
<222> (1)...(147)
<223> n=A,T,C or G

```

```

<400> 225
ttggacttct agactcacct gttctcactc cctgnttnaa ttnaaccag ncatgcaatg 60
ccaaataata naattgctcc ctaccagctg aacagggagg agtctgtgca gttinctgaca 120
cttggtgttg aacatgggta aatacaa                                     147

```

```

<210> 226
<211> 104
<212> DNA
<213> Homo sapiens

```

```

<220>
<221> misc_feature
<222> (1)...(104)
<223> n=A,T,C or G

```

```

<400> 226
nncaggnaca tgtgtgaaaa caatattgta tactaccata gtgagccatg antntntaaa 60
aaaaaaataa atgttttggg ggngatntgt attctccaac ttgg                                     104

```

```

<210> 227
<211> 491
<212> DNA
<213> Homo sapiens

```

```

<400> 227
acactgttgg tggtatatgg ggatgggggt ctcggtaatt ttgtttatta tttatgttta 60
ttattatggt ttatcattaa ttattcaata aatttttatt taaaaagtcg ccctacttag 120
aaatcttctg tgggggtggg agggacaaaa gattacaaac caaaactcag gagatggtaa 180
cactggaatt gataaaatca cctgggatta gtcgtataac tctgaaccac caaacctctg 240
ctatcaagcc ttgctacagt catggctgtc cagaaagatt tacagttatt tttctgagaa 300
aggatccatg ggctttaaga acttcagaac tttaagaact tcagaagttc ttaagttgct 360
gaagctcaag taacgaagt gaatgcaatc aaaaaaagaa taccagggag tcaaggcttg 420
agaggcacat tcttatccta aagtgactgc tcaaacctga cgagaccaag taaattactg 480
aagatacaaa g                                     491

```

```

<210> 228
<211> 328
<212> DNA
<213> Homo sapiens

```

```

<400> 228
actcagcgcc agcatcgccc cacttgattt tggagggatc tcgctcctgg aagatgggtga 60
tgggatttcc attgatgaca agcttcccg tctcagcett gacggtgcca tgggaatttgc 120

```

```
catgggtgga atcatattgg aacatgtaa ccatgtagtt gaggtcaatg aaggggtcat 180
tgatggcaac aatatccact ttaccagagt taaaagcagc cctggtgacc aggcgccccaa 240
tacgacaaaa tccgttgact ccgaccttca ccttccccat ggtgtctgag cgatgtggct 300
cggctggcga cgaaaaagaa gatgcggc 328
```

<210> 229  
<211> 689  
<212> DNA  
<213> Homo sapiens

<220>  
<221> misc\_feature  
<222> (1)...(689)  
<223> n=A,T,C or G

```
<400> 229
accacagcat catcccttgg tccagaatct actaccttcc acagcggccc aggtccact 60
gaaacaacac tcctacctga caacaccaca gcctcaggcc tccttgaagc atctacgccc 120
gtccacagca gcactggatc gccacacaca acactgtccc ctgccggntc tacaacccgt 180
cagggagaat ctaccacctt ccagagctgg ccaaactcga aggacactac ccctgcacct 240
cctactacca catcagcctt tgttgagcta tctacaacct cccacggcag cccgagctca 300
actccaacaa cccacttttc tgccagctcc acaaccttgg gccgtagtga ggaatcgaca 360
acagttccaca gcagcccagt tgcaactgca acaacacctt cgcttgccca ctccacaacc 420
tcaggcctcg ttgaagaatc tacgacctac cacagcagcc cgggctcaac tcaaacaatg 480
cacttccctg aaagcgacac aacttcaggc cgtggtgaag aatcaacaac ttcccacagc 540
agcacaacac acacaatatc ttcagctcct agcaccacat ctgcccttgt tgaagaacct 600
accagctacc acagcagccc gggctcaact gcaacaacac acttcccttg acaggttcca 660
caacctcaag gccgtagtgg agggaaatc 689
```

<210> 230  
<211> 483  
<212> DNA  
<213> Homo sapiens

```
<400> 230
gggttctagc tcctccaatc ccattttatc ccatggaacc actaaaaaca aggtctgctc 60
tgctcctgaa gccctatatg ctggagatgg acaactcaat gaaaatttaa agggaaaacc 120
ctcaggcctg aggtgtgtgc cactcagaga cttcacctaa ctagagacag gcaaactgca 180
aaccatgggt agaaattgac gacttcacac tatggacagc ttttcccaag atgtcaaaac 240
aagactcctc atcatgataa ggctcttacc cccttttaac ttgtccttgc ttatgcctgc 300
ctctttcgct tggcaggatg atgctgtcat tagtatttca caagaagtag cttcagaggg 360
taacttaaca gagtgtcaga tctatcttgt caatcccaac gttttacata aaataagaga 420
tccttttagt caccagtgca ctgacattag cagcatcttt aacacagccg tgtgttcaaa 480
tgt 483
```

<210> 231  
<211> 447  
<212> DNA  
<213> Homo sapiens

```
<400> 231
accctctcta ttcactagct tctgaaaagg gaggagtatt tttagtttga caatttaata 60
atttaaaaac aagacatctc caggtaggaa aaaatgaaag ctatttcatg caaacattat 120
ctaatttagc ttaaaagtga aagtggtaat actgttggtt tctgtaaatg ttgcagggtt 180
ttaaacttta taattacttt aatatttttg ataactagaa atctagtatt gccataaagg 240
aaactaagtg cccatcaaag atttgttttg tataaataaa gaattatttg ttttgttttc 300
aatgacagta agctacaaat catgatgctt aaaaactttc taaagatgaa ttgtgtggca 360
gtgattggtc tgtttgtgga gaattgtatga aagctattaa tattctagaa tagattaata 420
```



aattggctat gttgttccaa tgaatgt

447

&lt;210&gt; 232

&lt;211&gt; 649

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (1)...(649)

&lt;223&gt; n=A,T,C or G

&lt;400&gt; 232

```

gtgggcagaa gaaaaagcta gtgatcaaca gtggcaatgg agctgtggag gacagaaagc 60
caagtggact caacggagag gccagcaagt ctcaggaaat ggtgcatttg gtgaacaagg 120
agtcgtcaga aactccagac cagtttatga cagctgatga gacaaggaaac ctgcagaatg 180
tggacatgaa gattgggggtg taacacctac accattatct tggaaagaaa caaccgttgg 240
aaacataacc attacaggga gctgggacac ttaacagatg caatgtgcta ctgattgttt 300
cattgcgaat ctttttttagc ataaaaatct ctattccttt tgttttttgt gttttgttct 360
ttaaagtcag gtccaatttg taaaaacagc attgctttct gaaattaggg cccaattaat 420
aatcagcaag aatttgatcg ttccagttcc cacttggagg cctttcatcc ctcggtgtg 480
ctatggatgg cttctaacaa aaactacaca tatgtattcc tgatcgccaa cctttccccc 540
accagctaag gacatttccc agggttaata gggcctggtc cctgggagga aatttgaatg 600
ggtccatttt gcccttncat agcctaattcc ctgggcattg ctttncact 649

```

&lt;210&gt; 233

&lt;211&gt; 396

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 233

```

acaatgcaaa acataagtaa tcttttcact attataacac ttgtatgatt ttaagacaaa 60
cttggcttaa attaagtttt ggggtcagcc ccaaattcct gcccttcac tgtattttga 120
attattttta aactctcaga tacagcttta tagttaaaac attattagac tatatattct 180
aaattctaaa gtgaccaaag gggacagttt atgtaaagat aacacttttt cttaattttt 240
agaaaacccat tctttcatct cctggtggtc ttctttttcc gtctctatct cttttgtag 300
catcctatct ggtagtgtgt taatatacat cttccctgag tgtttttaca acacaaagcc 360
atttagtgat tctgaatggc tactctgcct gccagt 396

```

&lt;210&gt; 234

&lt;211&gt; 4627

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 234

```

tcacttgccat gatattttcca gtgtcagagg gacacagcca acgtgggggc ccttctaggc 60
tgacagccgc tctccagcca ctgccgagag ccgctctgct ccgcccctgc ccgtgcactc 120
tccgcagccg cctccgcca agccccagcg ccgctccca tcgccgatga ccgcggggag 180
gaggatggag atgctctgtg ccggcagggg cctgcgctg ctgctctgcc tgggtttcca 240
tcttctacag gcagtcctca gtacaactgt gattccatca tgtatcccag gagagtccag 300
tgataactgc acagcttttag ttccagacaga agacaatcca cgtgtggctc aagtgtcaat 360
aacaagtgt agctctgaca tgaatggcta ttgtttgcat ggacagtgc tctatctggt 420
ggacatgagt caaaactact gcaggtgtga agtgggttat actggtgtcc gatgtgaaca 480
cttcttttta accgtccacc aacctttaag caaagagtat gtggctttga ccgtgattct 540
tattattttg tttcttatca cagtcgtcgg ttccacatat tattctgca gatggtacag 600
aaatcgaaaa agtaaagaac caaagaagga atatgagaga gttacctcag gggatccaga 660

```

```

gttgccgcaa gtctgaatgg cgccatcaaa cttatgggca gggataacag tgtgcctggt 720
taatattaat attccatttt attaataata tttatgttgg gtcaagtgtt aggtcaataa 780
cactgtattt taatgtactt gaaaaatgtt tttatttttg ttttattttt gacagactat 840
ttgctaagtgt ataatgtgca gaaaatattt aatatcaaaa gaaaattgat atttttatac 900
aagtaatttc ctgagctaaa tgcttcattg aaagcttcaa agtttatatg cctgggtgcac 960
agtgcttaga agtaagcaat tcccagggtca tagctcaaga attgttagca aatgacagat 1020
ttctgtaagc ctatatatat agtcaaatcg atttagtaag tatgtttttt atgttctcca 1080
aatcgatgat aattggtttg actgtaccat ggtttgatat gtagttggca ccatgggtac 1140
atatattaaa acaataatgc aattagaatt tgggagaagc aaatataggt cctgtgttaa 1200
acactacaca ttgaaacaa gctaaccctg gggagtctat ggtctcttca ctcagggtctc 1260
agctataatt ctgttatatg aggggagctg gacagttccc tatgccaaact cacgactcct 1320
acaggtagta gtccactcatc taccagattc tgccatgtat aaatgaattg aaaaacaata 1380
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cttgaagcct aaatttgtgc tttttaagaa tattttttaga ctatttcttt ttataggggc 1560
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aatatatgaa gacaataatt ctacatgttg tcttaagatg gaaatacagt tatttcatct 1680
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agttatgtat ttaaagttgt atcttgacac aggaaatggg aaaaaactta aaaattaata 1800
tggtgtattt ttccaaatga aaaaatctcaa ttgaaagcct ttaaaatgta gaaacttaaa 1860
cacaccttcc tgtggaggct gagatgaaaa ctagggtcct ttttctgac atttgtttat 1920
tttttgggag agacaaagat ttcttctgca ctctgagccc atagggtctca gagagttaat 1980
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tcaggaggga tctgttcttc ctctacgttt atcctggcat gtgctagggt aaacgaaggc 2160
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catgcattat taaccctggg gcaatcacac gactgtcatc taaagtctg gccctggccc 2280
ttactattag gaaaataaac agacaaaaac aagtaaatat atatggtcct atacatatg 2340
tatatatatt catatacaaa catgtatgta tacatgacct taatggatca tagaattgca 2400
gtcatttggg gcctctgctaa ccatttatat aaaacttaaa aacaagagaa aagaaaaatc 2460
aattagatct aacacgtttt ttctgtttcc tatttaatat agctgaagtc aaatgatga 2520
agaacacatt ttaaatactc tacttacagt tggccctctg tggttagttc cacatctgtg 2580
gattcaacca accaaggacg gaaaaatgctt aaaaaataat acaacaacaa caaaaaatc 2640
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tctgtgcccc atttttagtat gtgcaggggg gcgatctggg aatcagtccc ctgtggatac 3120
caaggtacaa ctgtattttat taacgcttac tagatgtgag gagagtctga atattttcag 3180
tgatcttggc tgtttcaaaa aaatctattg acttttcaat aaatcagctg caatccattt 3240
atttcattta caaaagattt attgtaagcc tctcaatctt ggtttttcag ttgatcttaa 3300
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aaggggctat gcatattcaa tgtattgaga accaaagcaa ccacaaatgc ataaatgcat 3660
aatttatggt ctccaaccaa ggccacataa taaccagtt aacttactct ttaaccagga 3720
atattaagtt ctataactag tactcaaggt ttaaccttaa aattaagatt tccttaacct 3780
taaccttaaa attgatatta tattaacat acataataca atgtaactcc actgttctcc 3840
tgaattttt ttgctctaatt ctctctgccg aaagtcaaag tgatgggaga attggtatac 3900
tggtatgact acgtcttaag atttatgagt atttatgagt ctttgagact aaattcaatc 3960
accaccaggt atcaaatcaa cttttatgca gcaaatatat gattctagtg tctgactttt 4020
gttaaatcca gtaatgcagt ttttaaaaaa ctgtatctga cccactttgt aatttttgct 4080
ccaatatcca ttctgtagac ttttgaaaaa aaagttttta atttgatgcc caatatattc 4140

```

```

tgaccggttaa aaaattcttg ttcatatggg agaaggggga gtaatgactt gtacaaacag 4200
tatttctgggt gtatatattta atgtttttta aaagagtaat ttcattttaa tatctgttat 4260
tcaaatttga tgatgttaaa tgtaataata tgtattttct ttttattttg cactctgtaa 4320
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aacataaagt tgtattgcat gcaattttaa gtaacttatt tgactatgaa tattatcgga 4440
ttactgaatt gtatcaattt gtttgtgttc aatatcagct ttgataattg tgtaccttaa 4500
gatattgaag gagaaaatag ataatttaca agatattatt aatttttatt tatttttctt 4560
gggaattgaa aaaaattgaa ataaataaaa atgcattgaa catcttgcac tcaaaatctt 4620
cactgac 4627

```

&lt;210&gt; 235

&lt;211&gt; 169

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 235

```

Met Thr Ala Gly Arg Arg Met Glu Met Leu Cys Ala Gly Arg Val Pro
          5                      10                      15

```

```

Ala Leu Leu Leu Cys Leu Gly Phe His Leu Leu Gln Ala Val Leu Ser
          20                      25                      30

```

```

Thr Thr Val Ile Pro Ser Cys Ile Pro Gly Glu Ser Ser Asp Asn Cys
          35                      40                      45

```

```

Thr Ala Leu Val Gln Thr Glu Asp Asn Pro Arg Val Ala Gln Val Ser
          50                      55                      60

```

```

Ile Thr Lys Cys Ser Ser Asp Met Asn Gly Tyr Cys Leu His Gly Gln
          65                      70                      75                      80

```

```

Cys Ile Tyr Leu Val Asp Met Ser Gln Asn Tyr Cys Arg Cys Glu Val
          85                      90                      95

```

```

Gly Tyr Thr Gly Val Arg Cys Glu His Phe Phe Leu Thr Val His Gln
          100                      105                      110

```

```

Pro Leu Ser Lys Glu Tyr Val Ala Leu Thr Val Ile Leu Ile Ile Leu
          115                      120                      125

```

```

Phe Leu Ile Thr Val Val Gly Ser Thr Tyr Tyr Phe Cys Arg Trp Tyr
          130                      135                      140

```

```

Arg Asn Arg Lys Ser Lys Glu Pro Lys Lys Glu Tyr Glu Arg Val Thr
          145                      150                      155                      160

```

```

Ser Gly Asp Pro Glu Leu Pro Gln Val
          165

```

&lt;210&gt; 236

&lt;211&gt; 894

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 236

```

atgcacacc atcaccatca cacggccgcg tccgataact tccagctgtc ccagggtggg 60
cagggttcg ccattccgat cgggcaggcg atggcgatcg cgggccagat caagcttccc 120

```

```

accgttcata tcgggcctac cgccttcctc ggcttgggtg ttgtcgacaa caacggcaac 180
ggcgcacgag tccaacgcgt ggtcgggagc gctccggcgg caagtctcgg catctccacc 240
ggcgacgtga tcaccgcgt cgacggcgct ccgatcaact cggccaccgc gatggcggac 300
gcgcttaacg ggcatcatcc cgggtgacgtc atctcgggtga cctggcaaac caagtccggc 360
ggcacgcgta cagggaaacgt gacattggcc gagggacccc cggccgaatt cgaatgccttc 420
ctgaaatatg agaaggccga caaatactac tacacaagaa aatgtcgcaa tctgtgtgcc 480
ttcctgaggg gcaacctgctc attttgcagc cgcacactga gaaagcaatt ggatcacaa 540
ctcaccttcc acaagctggg ggcctatatg atctgcctac atacagctat tcacatcatt 600
gcacacctgt ttaactttga ctgctatagc agaagccgac aggccacaga tggctccctt 660
gcctccattc tctccagcct atctcatgat gagaaaaagg ggggttcttg gctaaatccc 720
atccagtcoc gaaacacgac agtggagtat gtgacattca ccagccgggg tcaaacagag 780
gagagcatga atgagagtca tcctcgcaag tgtgcagagt cttttgagat gtgggatgat 840
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```

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&lt;211&gt; 297

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 237

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&lt;210&gt; 245

&lt;211&gt; 564

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 245

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
20 December 2001 (20.12.2001)

PCT

(10) International Publication Number  
**WO 01/096390 A3**

- (51) International Patent Classification<sup>7</sup>: C07K 14/47, 16/18, C12N 5/06, 15/12, 15/62, C12Q 1/68, G01N 33/68, A61K 31/7088, 35/14, 38/17, 39/395
- (74) Agents: POTTER, Jane, E., R.; Seed Intellectual Property Law Group PLLC, Suite 6300, 701 Fifth Avenue, Seattle, WA 98104-7092 et al. (US).
- (21) International Application Number: PCT/US01/18577
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (22) International Filing Date: 8 June 2001 (08.06.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
60/210,821 9 June 2000 (09.06.2000) US  
60/256,571 18 December 2000 (18.12.2000) US  
60/290,240 10 May 2001 (10.05.2001) US
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (*for all designated States except US*): CORIXA CORPORATION [US/US]; 1124 Columbia Street, Suite 200, Seattle, WA 98104 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): JIANG, Yuqiu [CN/US]; 5001 S. 232nd Street, Kent, WA 98032 (US). HEPLER, William, T. [US/US]; 12034 38th Avenue N.E., Seattle, WA 98125 (US). CLAPPER, Jonathan, D. [US/US]; 2149 Dexter Avenue N., #4, Seattle, WA 98109 (US). WANG, Aijun [CN/US]; 3106 213th Place S.E., Issaquah, WA 98029 (US). SECRIST, Heather [US/US]; 3844 35th Avenue W., Seattle, WA 98199 (US).
- Published:  
— with international search report
- (88) Date of publication of the international search report:  
27 December 2002
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF COLON CANCER

(57) Abstract: Compositions and methods for the therapy and diagnosis of cancer, such as colon cancer, are disclosed. Compositions may comprise one or more colon tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a colon tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as colon cancer. Diagnostic methods based on detecting a colon tumor protein, or mRNA encoding such a protein, in a sample are also provided.

WO 01/096390 A3

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 01/18577

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/47 C07K16/18 C12N5/06 C12N15/12 C12N15/62  
C12Q1/68 G01N33/68 A61K31/7088 A61K35/14 A61K38/17  
A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 28031 A (UNIV EMORY) 18 May 2000 (2000-05-18) see mitogenic regulator mox1 and passages relating thereto page 9, line 21; examples 1,2,9 page 10, line 16 - line 17 ---	1-17
X	WO 99 60161 A (DIADEXUS LLC ; SUN YONGMING (US); YANG FEI (US); MACINA ROBERTO A ( ) 25 November 1999 (1999-11-25) see seq. ID.3 --- -/-	1-17

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
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- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

24 July 2002

Date of mailing of the international search report

07. 08. 2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Smalt, R

## INTERNATIONAL SEARCH REPORT

In International Application No

PCT/US 01/18577

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBL 'Online!  Entry HS573344, Acc.no. W37573,  17 March 1996 (1996-03-17)  HILLIER, L. ET AL.: "zc10c08.r1  Soares_parathyroid_tumor_NbHPA Homo  sapiens cDNA clone IMAGE:321902 5', mRNA  sequence."  XP002194983  the whole document</p> <p>---</p>	1-5,8,11
X	<p>DATABASE EMBL 'Online!  Entry/Acc.no. AP001336,  6 March 2000 (2000-03-06)  HATTORI, M. ET AL.: "Homo sapiens genomic  DNA, chromosome 18q12 clone:RP11-735F7,  WORKING DRAFT SEQUENCE, 18 unordered  pieces."  XP002194984  see nt. 195415-195748</p> <p>---</p>	1,11
A	<p>YAMAMOTO M ET AL: "RAISED  PROSTATE-SPECIFIC ANTIGEN IN  ADENOCARCINOMA OF THE COLON"  INTERNATIONAL UROLOGY AND NEPHROLOGY,  AKADEMIAI, BUDAPEST, HU,  vol. 29, no. 2, 1997, pages 221-225,  XP000889898  ISSN: 0301-1623</p> <p>---</p>	
A	<p>WO 99 62942 A (SAFFRAN DOUGLAS C ;AFAR  DANIEL E (US); HUBERT RENE S (US); LEONG  K) 9 December 1999 (1999-12-09)</p> <p>---</p>	
A	<p>EP 0 628 820 A (MILES INC)  14 December 1994 (1994-12-14)</p> <p>---</p>	
P,X	<p>WO 01 22920 A (HUMAN GENOME SCIENCES INC  ;ROSEN CRAIG A (US); BARASH STEVEN C (US)  5 April 2001 (2001-04-05)  see seq.ID.962, 2226 and 6503</p> <p>---</p>	1-8, 11-17
E	<p>WO 01 96388 A (HARLOCKER SUSAN L ;CORIXA  CORP (US); JIANG YUQIU (US); SECRIST HEA)  20 December 2001 (2001-12-20)  See seq.ID.1170 (and others).</p> <p>---</p>	1-17
E	<p>WO 01 49716 A (MEAGHER MADELEINE JOY  ;CORIXA CORP (US); JIANG YUQIU (US); KING  GO) 12 July 2001 (2001-07-12)  See seq.ID.803</p> <p>---</p>	1-17
E	<p>WO 01 73027 A (MEAGHER MADELEINE JOY  ;CORIXA CORP (US); KING GORDON E (US); XU  JI) 4 October 2001 (2001-10-04)  see seq.ID.38</p> <p>---</p>	1-17
	-/--	

## INTERNATIONAL SEARCH REPORT

Int. lional Application No

PCT/US 01/18577

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 02 06515 A (DIADEXUS INC ;SUN YONGMING (US); MACINA ROBERTO A (US)) 24 January 2002 (2002-01-24) the whole document ----	1-17
E	WO 02 12328 A (MEAGHER MADELEINE JOY ;CORIXA CORP (US); KING GORDON E (US); SECRI) 14 February 2002 (2002-02-14) see seq.476 ----	1-17
E	WO 01 55314 A (HUMAN GENOME SCIENCES INC ;ROSEN CRAIG A (US); BARASH STEVEN C (US) 2 August 2001 (2001-08-02) see seq.ID.59 and passages related thereto -----	1-5,8, 11,12, 14-16

## INTERNATIONAL SEARCH REPORT

ational application No.  
PCT/US 01/18577

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Although claims 12,13 and 17, and claim 9, in as far as it pertains to in vivo use, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:  
1-17, all partially
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1-17, all partially

Isolated polynucleotide according to seq.ID.1, homologues with at least 75% identity, complements or fragments thereof, polypeptides encoded thereby, expression vector, host cell, antibody, methods for diagnosing cancer and kits for use therein, fusion protein, hybridizing oligonucleotide, method for stimulating T cells and T cell population obtained thereby, compositions of the proteins, nucleic acids, antibodies or an antigen presenting cell.

Inventions 2-236: claims 1-17, all partially

Subject matter as defined for the first invention, but limited to the respective seq.ID's 2-234, 236 and 244, whereby invention 2 relates to seq.ID.2, invention 3 to seq.ID.3, ...invention 234 to seq.ID.234, invention 235 to seq.ID.236, and invention 236 to seq.ID.244.

For the sake of conciseness, the first subject matter is explicitly defined, the other subject matters are defined by analogy thereto.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 01/18577

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

In International Application No  
PCT/US 01/18577

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WO 0155314 A		AU 3795001 A	07-08-2001
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